LEABHARLANN CHOLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH | TRINITY COLLEGE LIBRARY DUBLIN
Ollscoil Átha Cliath | The University of Dublin

Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

A Study of the Multiple Functions and Interactions of Semicarbazide Sensitive Amine Oxidase

Jeffrey O'Sullivan 2005 PhD

A Study of the Multiple Functions and Interactions of Semicarbazide Sensitive Amine Oxidase

by

Jeffrey O'Sullivan

A dissertation submitted to the University of Dublin, Trinity College in candidature for the degree of Doctor of Philosophy

Department of Biochemistry
Trinity College
Dublin 2
Ireland

June 2005

TRINITY COLLEGE
10 JUL 2006
LIBRARY DUBLIN
THESIS
7925

Table of Contents

	Page Number
Declaration	iii
Acknowledgements	iv
Summary	v
Table of Abbreviations	vii
Detailed List of Contents	1
Chapter 1	
Introduction to Amine Oxidases	8
Chapter 2	
General Methods	70
Chapter 3	
Interaction between SSAO/VAP-1 and amino hexoses: implications for the modulation of cell adhesion	103
Chapter 4	
Inhibition of amine oxidises by the histamine-1 receptor antagonist hydroxyzine and a possible role in multiple sclerosis	160
Chapter 5	
Metabolism of endogenous amine oxidase substrates and their role in glucose uptake by adipocytes	215
Chapter 6	
General Discussion	273
Appendix	283
Bibliography	285
Publications	322

Declaration

I certify that none of the work presented in this thesis has been submitted for any degree at this, or any other University and that the work described in this thesis is entirely my own.

I agree that the Library may lend or copy this thesis upon request.

Jeffrey O'Sullivan

Acknowledgements

Firstly I would like to thank my supervisor. Prof. Keith Tipton for allowing me the opportunity to work along side him and for the endless advice, encouragement and of course that unique sense of humour.

A big thanks to the fellow members of Lab One, past and present. To Gavin for taking the years of ridicule in good spirits (most of the time!), to Martha for the years of looking after us all, to Joe, what can I say the road is long but we've made it, to Andrew for all things Mac (but apologies, this thesis was compiled in WoRd!), to Sinead for the use of the office, to Aoife for the endless banter and supply of rats, to the new Lab One wannabes, when are you moving and where are my pipettes!

Thanks also to Prof. Mercedes Unzeta and Dr. Laura Raimondi for allowing me to spend some time in their labs in Barcelona and Florence. Great people, great places, great food and even some science!

To the lads and lassies in the other labs around the department most notably the three stooges belonging to the Mantle Lab, Gav, Ed and Shea, nearly there lads!

For the all important financial assistance throughout my studies I wish to thank the Health Research Board Ireland (HRB), Enterprise Ireland, the Irish Society of Toxicology and County Dublin VEC.

To my friends and family (especially my sister Lisa for moving out and giving me some peace and quiet in which to study!), yes finally I'll no longer be pulling out the student card for the cheap deals!!!

For Barbara for sticking with me through the many years of college and putting up with all the science stuff, your turn now!

To my parents Hilda & Denis for supporting and encouraging me throughout my college career and yes I did take the scenic route but I promise now I'll get a proper job. Finally to my Gran for just being there, I dedicate this thesis to you.

Summary

This thesis focuses on three of the recognised multiple functions of mammalian semicarbazide sensitive amine oxidase (SSAO) and the contribution of monoamine oxidase (MAO) to some of these.

SSAO is a vascular-adhesion protein, under the guise vascular adhesion protein-1 (VAP-1). As many cell-cell interactions occur by specific recognition of carbohydrate ligands, the interactions between monosaccharide derivatives and SSAO were studied. Mannosamine, glucosamine and galactosamine were found to be time-dependent inhibitors of SSAO activity, whereas their *N*-acetyl derivatives and the unsubstituted hexoses failed to inhibit the enzyme. Differences in potency displayed by amino sugars suggest specificity towards the structure of sugar. The inhibition was concentration dependent but fully reversible. The H_2O_2 formed as a product of the SSAO-catalysed oxidation reaction was shown to be necessary to enable the inhibition of the enzyme by galactosamine. Several other compounds were tested as possible analogues, including serinol, pyrroline-5-carboxylic acid and three 2-amino sugar analogues. Inhibition was observed in some cases but this was was not time dependent.

SSAO is known to be a binding protein for drugs, such as amiloride and some other imidazoline and guanidine compounds Hydroxyzine, a drug with a different spectrum of actions from amiloride, was also shown to be an inhibitor of SSAO and MAO. Both plasma SSAO and MAO were inhibited in a freely reversible and competitive manner by hydroxyzine. However, the membrane-bound form of SSAO was found to be significantly less sensitive to inhibition (IC_{50} values of ~20 μM for plasma SSAO compared to 1 mM for the membrane-bound form).

The reduction observed in mast cell activation by hydroxyzine treatment in C57 black mice induced with experimental allergic encephalomyelitis (EAE) suggested a possible role for SSAO in the progression of EAE. The 'suicide' inhibitor of SSAO 2-bromoethylamine was shown to decrease the SSAO activity after administration to C57 black mice. Histological analysis of brain tissue from both treated and untreated mice

showed a marked effect on the progression of EAE. Since, 2-bromoethylamine is a relatively weak SSAO inhibitor with an intrinsic reactivity that may result in other interactions within the tissues, the effects of the more potent SSAO inhibitor, methylhydrazine, were investigated for comparison. However, there was an unexpected high mortality rate in the mice treated with this compound, which prevented a thorough examination of any effects that it had on the progression of EAE in this model.

SSAO is co-localised with the glucose transporter (GLUT4) in adipocytes and when adipocytes are exposed to an amine substrate the hydrogen peroxide formed stimulates an increase in glucose transport. As both physiological and non-physiological amines stimulate an increase of glucose transport, the possibility that adrenaline and noradrenaline can, either in conjunction with or via a distinct mechanism from the adrenoceptor-dependent pathway, can regulate the translocation of GLUT 4, to enable an increase in glucose uptake. Adrenaline, which is oxidised by MAO to form methylamine, which is a substrate for SSAO in both the MAO- and SSAO-catalysed reactions was shown to increase glucose uptake, presumably *via* GLUT4 mobilisation. In contrast, stimulation of glucose uptake in the presence of noradrenaline is mediated by MAO alone.

Abbreviations

2-BEA	2-Bromoethylamine
2-PEA	2-Phenylethylamine
3-D	Three dimensional
5-HT	5-hydroxytryptamine (serotonin)
AD	Alzheimer's disease
AGE	Advanced glycation end product
ALDH	Aldehyde dehydrogenase
AMP	Adenosine monophosphate
AMPK	AMP activated protein kinase
AR	Aldose or aldehyde reductase
ATP	Adenosine triphosphate
BAO	Benzylamine oxidase
BAT	Brown adipose tissue
β-APN	Beta-aminopropionitrile
BSA	Bovine serum albumin
bSSAO	Bovine semicarbazide sensitive amine oxidase
c.p.m.	Counts per minute
CAM	Cell adhesion molecule(s)
cAMP	Cyclic adenosine monophosphate
cDNA	Complmentary deoxyribonucleic acid
CHD	Coronary heart disease
CHF	Congestive heart failure
CHO	Carbohydrate
Ci	Curie (2.22×10^{12} dpm)
CNS	Cetral nervous system
Con-A	Conconavlin-A
Da	Dalton
DA	Dopamine
DAO	Diamine oxidase
DβH	Dopamine β-hydroxylase
DHMA	3,4-Dihydroxymandelic acid
DNA	Deoxyribonucleic acid
DOPA	3,4-Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic acid
DOPAL	3,4-Dihydroxyphenylacetaldehyde
DOPET	3,4-Dihydroxyphenylethanol
d.p.m	Disintegrations per minute
DSA	Diazotised sulphanilic acid
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraactic acid
EGTA	ethylene glycol bis(2-aminoethyl ether)-N,N,N'N'-tetraacetic acid

F6P	Fructose-6-phosphate
FAD	Flavin-adenine dinucleotide
$FADH_2$	Flavin-adenine dinucleotide, reduced form
FDH	Formaldehyde dehydrogenase
FPFA	4-fluorophenethyl)-3-fluoroallylamine
g	acceleration due to gravity
GAD	Generalised anxiety disorder
GAG	Glycosaminoglycan(s)
Gal	Galactosamine
GLP-1	Glucagon-like peptide-1
Glu	Glucoamine
GLUT	Glucose transporter
HAT	Hydrogen atom transfer
hCAO	Human copper amine oxidase
hCG	Human chorionic gonadotropin
HEV	High endothelial venules
HMIT	H^+-coupled myo-inositol transporter
HPAO	Hansulella polymorpha amine oxidase
HRP	Horse radish peroxidase
HVA	Homovanillic acid
HVAP-1	Human vascular adhesion protein-1
IC_{50}	Inhibitor concentration giving 50% reduction in enzyme activity
ICAM	Intracellular adhesion molecule
Ig	Immunoglobulin
IL	Interlukin
INF	Interferon gamma
IRS	Insulin receptor substrates
k_{app}	*Pseudo* first-order rate constant
K_i	Inhibitor constant
K_m	Michaelis constant
LO	Lysyl oxidase
LPS	Lipopolysaccharide
Man	Mannosamine
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MAPK	Mitogen activated protein kinase
MBS	Metal binding site
MOG	Myelin oligodendrocyte glycoprotein
$MPDP^+$	1-Methyl-4-phenyl-2,3-dihydropyridinium
MPP^+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	Messenger ribonucleic acid
MS	Mass spectroscopy
MS	Multiple sclerosis
mSSAO	Mouse semicarbazide sensitive amine oxidase
NAD^+	Nicotinamide-adenine dinucleotide, oxidised form

NADH	Nicotinamide-adenine dinucleotide, reduced form
NDP	Norrie disease gene
NMR	Nuclear magnetic resonance
PAO	Polyamine oxidase
PBS	Phosphate buffer saline
PD	Parkinsons disease
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PLP	Pyridoxal phosphate
PNMT	phenolethanolamine-*N*-methylatransferase
PPO	2,5-diphenyl oxazole
PQQ	Pyrroloquinoline quinone
PTS	Phosphoeynolpyruvate phosphotransferase system
ROS	Reactive oxygen species
S.A.	Specific activity
S.E.M.	Standard error of the mean
SDAT	Senile dementia of Alzheimer's type
SDS	Sodium dodecyl sulphate+B11
SDS-PAGE	Sodium dodecyl sulphate polyacrylaminde-gel electrophoresis
SET	Single electron transfer
SGLT1	Sodium-glucose cotransporter gene-1
SMC	Smooth muscle cells
SSAO	Semicarbazide sensitive amine oxidase
TBS	Tris buffered saline
TEMED	N,N,N,N-tetra-ethylenemethylenediamine
TIL	Tumor infiltrating lymphocytes
TNF-α	Tumour necrosis factor alpha
TOPA	3,4,6-Trihydroxyphenylalanine
TPQ	TOPA quinone
Tris	Tris (hydroxymethyl) aminomethane
TTBS	Tween-tris buffered saline
U	Unit of enzyme activity (usually 1 μmol product.min^{-1})
UCP1	Uncoupling protein 1
UV-VIS	Ultra violet-visible
v	Intial velocity of an enzyme-catalysed reaction
v/v	volume by volume
VAP-1	Vascular adhesion pretin-1
V_{max}	Maxium velocity of an enzyme-catalysed reaction
w/v	weight by volume
WAT	White adipose tissue

Detailed list of contents

CHAPTER ONE 8

INTRODUCTION TO AMINE OXIDASES 8

1.1 A BRIEF HISTORY OF AMINE OXIDASES 9

1.1.2 Modern Nomenclature of amine oxidases. 11

1.2 MONOAMINE OXIDASES (MAO) 12

1.2.1 Sequence analysis of MAO 21

1.2.2 THE GENETICS OF MONOAMINE OXIDASE 23

1.2.3. Primary function of MAO 24

1.2.4. MAO pathology 27

1.2.5. MAO inhibitors 37

1.3. SEMICARBAZIDE SENSITIVE AMINE OXIDASE (SSAO) 41

1.3.1 Structure of SSAO 41

1.3.2. Organic co-factors 49

1.3.3. Reaction and mechanism 50

1.3.4 SSAO Substrates 51

1.3.5 SSAO Inhibitors 52

1.3.6 Distribution of SSAO 53

1.3.7 Pathology of SSAO 53

1.3.8 Multiple functions of SSAO 54

1.5 AIMS OF THESIS 68

CHAPTER 2 70

GENERAL METHODS 70

2.1.1 Source of reagents 72

2.2 PREPARATION OF SOLUTIONS 74

2.3 PROTEIN DETERMINATION 74

2.3.1 Biuret Assay 74

2.3.2 Markwell method 76

2.3.3 Bio-Rad protein assay 78

2.4 ENZYME ACTIVITY ASSAYS 79

2.4.1 Spectrophotometric assay of the deamination of benzylamine by SSAO 79

2.4.2 Spectrophotometric assay of the deamination of methylamine by SSAO 79

2.4.3 Hydrogen Peroxide Detection 80

2.4.4 Calculation of radiochemical concentrations 82

2.4.5 Radiochemical assay for SSAO activity 83

2.4.6 Radiochemical assay for MAO activity 84

2.4.7 Determination of the reversibility of SSAO inhibition 86

2.4.8 Determination of the reversibility of MAO inhibition 87

2.4.9 Curve fitting and statistical analysis 87

2.6 PREPARATION OF BOVINE LUNG MICROSOMES. 95

2.7 PREPARATION OF RAT LIVER MITOCHONDRIA 95

2.8 PREPARATION OF ADIPOCYTES FROM RAT WHITE ADIPOSE TISSUE 96

2.9 GLUCOSE UPTAKE DETERMINATION 96

2.10 ADIPOCYTE PLASMA MEMBRANE PREPARATION 97

2.11 RADIOCHEMICAL ASSAY FOR AMINE OXIDASE ACTIVITY IN ADIPOCYTES 99

2.12 CYTOLOGY 99

2.13 SODIUM DODECYL SULPHATE POLYACRYLAMIDE-GEL ELECTROPHORESIS (SDS-PAGE) 99

2.13.1 Sample preparation 99

2.13.2 Gel preparation and Electrophoresis 100

2.13.3 Staining and Destaining Gels 100

2.14 WESTERN BLOT ANALYSIS 101

CHAPTER 3 103

INTERACTION BETWEEN SSAO/VAP-1 AND AMINO HEXOSES: IMPLICATIONS FOR THE MODULATION OF CELL ADHESION. 103

3 INTRODUCTION 104

3.1 CELL TO CELL RECOGNITION 104

3.2 LEUKOCYTE MIGRATION 105

3.3 AMINO SUGARS 109

3.3.1 Amino sugar metabolism and catabolism 110

3.3.2 Roles of amino sugars 113

3.4 VAP-1/SSAO STORY 113

3.5 AIMS 116

3.7 RESULTS 120

3.7.1 The effect of physiological relevant amino hexes on the oxidative deamination of benzylamine by semicarbazide-sensitive amine oxidase (SSAO) 120

3.7.2 The effect of the amino hexoses, galactosamine and mannosamine on the oxidative deamination of benzylamine by semicarbazide sensitive amine oxidase (SSAO) 120

3.7.3 Effects of the products from the oxidative deamination of benzylamine by SSAO, on the inhibition of SSAO by galactosamine. 121

3.7.4 Removal of hydrogen peroxide by catalase abolishes the time-dependent inhibition of SSAO activity by galactosamine. 121

3.7.5 Determination of any possible synergism between galactosamine, hydrogen peroxide and benzylamine. 122

3.7.6 The effect of three structurally non-physiological amino hexose analogues on the oxidative deamination of benzylamine by SSAO. 122

3.7.7 Studies on the effects of other compounds with some structural features resembling those of the amino hexoses 122

3.7.8 Summary of the compounds in this study and their effect (if any) on the oxidative deamination of amine substrates by SSAO. 123

3.8 DISCUSSION 151

CHAPTER 4 160

INHIBITION OF AMINE OXIDASES BY THE HISTAMINE-1 RECEPTOR ANTAGONIST HYDROXYZINE AND A POSSIBLE ROLE IN MULTIPLE SCLEROSIS 160

4.1 ENZYME INHIBITION 161

4.2 CATALYTIC MECHANISMS OF THE AMINE OXIDASES 161

4.2.1 Monoamine oxidase 161

4.2.2 Semicarbazide-sensitive amine oxidase 164

4.3 AMINE OXIDASE INHIBITION 169

4.3.1 Reversible inhibitors of MAO 169

4.4.2 Irreversible inhibitors of MAO 170

4.4.3 Irreversible inhibitors of SSAO 170

4.5 HYDROXYZINE 171

4.6 MULTIPLE SCLEROSIS 174

4.7 AIMS & OBJECTIVES 176

4.8 MATERIALS AND METHODS 176

4.8 RESULTS 177

4.8.1 Preliminary determination of the effect of the histamine-1 receptor antagonist, hydroxyzine, on the oxidative deamination of benzylamine by bovine plasma SSAO. 177

4.8.2 Determination of the kinetic nature of the inhibition of bovine plasma SSAO by the histamine-1 receptor antagonist, hydroxyzine. 177

4.8.3 Effect of hydrogen peroxide on the inhibition of bovine plasma SSAO by the histamine-1 receptor antagonist, hydroxyzine. 177

4.8.5 Amine oxidase activity in isolated bovine lung microsomes. 178

4.8.6 Determination of the inhibition of isolated bovine lung microsomal SSAO by the histamine-1 receptor antagonist, hydroxyzine. 178

4.8.7 The effect of endoglycosidase treatment on activity and hydroxyzine mediated inhibition of partially purified bovine plasma SSAO 179

4.8.8 Determination of the inhibition of rat liver MAO A by the histamine-1 receptor antagonist, hydroxyzine. 179

4.8.9 Determination of the nature of the inhibition of rat liver MAO B by the histamine-1 receptor antagonist, hydroxyzine. 179

4.8.10 Determination of the reversibility of the inhibition on amine oxidases by the histamine-1 receptor antagonist, hydroxyzine. 180

4.8.11 Effect of the end products of MAO amine oxidation on the inhibition of bovine plasma SSAO by the histamine-1 receptor antagonist, hydroxyzine. 180

4.8.12 Inhibition mouse brain SSAO in encephalomyelitis -induced C57 black mice by 2-bromoethlyamine. 180

4.8.13 Effect of 2-bromoethlyamine on the onset of EAE-induced in C57 black mice. 181

4.8.14 Effect of methlyhydrazine on SSAO activity in plasma, lung and brain tissue from EAE-induced C57 black mice. 181

4.9 DISCUSSION 211

CHAPTER 5 215

METABOLISM OF ENDOGENOUS AMINE OXIDASE SUBSTRATES AND THEIR ROLE IN GLUCOSE UPTAKE BY ADIPOCYTES 215

5.1 INTRODUCTION 216

5.1 GLUCOSE METABOLISM 216

5.2 GLUCOSE IN HEALTH 217

5.2.1 Diabetes Mellitus 218

5.2.2 SSAO and Diabetes 219

5.2.3 Diabetes and Insulin in Alzheimer's Disease (AD) 221

5.2.4 Glycogen storage disease 223

5.2.5 Glucose-galactose malabsorption 223

5.2.6 Syndrome X 224

5.3 GLUCOSE TRANSPORT AND GLUCOSE TRANSPORTERS 224

5.3.1 Glucose transport 224

5.3.2 Glucose transporter proteins (GLUT) 225

5.3.3 Regulation of GLUT function 226

5.3.4 SSAO and glucose transporters 228

5.4Adipocytes 230

5.5 AIMS 233

5.7 RESULTS 236

5.7.1 Determination of total amine oxidase activity in adipocytes isolate from rat white adipose tissue. 236

5.7.2 Effects of amine oxidase inhibitors on basal glucose uptake in adipocytes isolated from rat white adipose tissue 236

5.7.3 Comparison of glucose uptake stimulation by insulin, (-)-adrenaline, (±)-adrenaline and noradrenaline. 236

5.7.4 Effects of monoamine oxidase inhibitors, clorgyline and deprenyl, on (±)- adrenaline, (-)-adrenaline and noradrenaline mediated glucose uptake in adipocytes isolated from rat white adipose tissue 237

5.7.5 Effects of the semicarbazide sensitive amine oxidase inhibitor, semicarbazide, on glucose uptake stimulated by the presence of (±)-adrenaline, (-)-adrenaline and noradrenaline in adipocytes isolated from rat white adipose tissue. 237

5.7.6 Effects of the combination of the amine oxidase inhibitors clorgyline, deprenyl and semicarbazide on (±)-adrenaline, (-)-adrenaline and noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue. 238

5.7.7 Effects of catalase on (±)-adrenaline, (-)-adrenaline and noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue. 238

5.7.8. Effects of DSA (Diazotised sulphanilic acid) on (±)-adrenaline, (-)-adrenaline and noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue. 239

5.7.9 Effects of vanadate on (±)-adrenaline, (-)-adrenaline and noradrenaline noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue. 239

5.7.10 Effects of of an adrenaline uptake inhibitor (-)-adrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue. 239

5.7.11 Effects of α-and β-adrenergic antagonists on (±)-adrenaline and noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue. 240

5.8 DISCUSSION 263

CHAPTER SIX 272

GENERAL DISCUSSION 272

6.1 DISCUSSION 273

6.2 FUTURE STUDIES 279

6.3 CONCLUDING REMARKS 281

APPENDIX 283

Appendix I 284

BIBLIOGRAPHY 285

PUBLICATIONS 322

Chapter One

Introduction to Amine Oxidases

1.1 A Brief history of amine oxidases

The notion of amine oxidases first appeared in 1877 when Schmiedeberg reported feeding benzylamine to dogs and noted the formation of hippuric acid in the dogs' urine. The logical conclusion from this observation was that an unknown component was metabolising the amine (Schmiedeberg, 1877). Amine oxidase research only reappeared 50 years later upon the discovery of an enzyme possessing the ability to oxidatively deaminate the amine tyramine (Hare, 1928). The enzyme in question became know as "tyramine oxidase". Following on from the discovery made by Hare an enzyme histaminase, which was capable of deaminating histamine in lung, liver and kidney, was discovered (Best, 1929). Ten years later Blaschko showed that the tyramine oxidase, which was also now known as monoamine oxidase (MAO), could act on catecholamines and other monoamines (Blaschko, 1937) Pugh and Quastel also showed the presence of the amine oxidising enzyme in rat and guinea pig brain (Pugh & Quastel, 1937).

Differences between the two enzymes, "tyramine oxidase" and "histaminase" began to come to light when differences in location and the level of inhibition caused to the enzymes by cyanide were reported. Further separation of the enzymes into unique proteins occurred when Zeller discovered that diamines such as putrescine and cadaverine which showed no interaction with "tyramine oxidase" were deaminated by the pig kidney "histaminase". A proposal was put forward renaming these enzymes "monoamine oxidase" and "diamine oxidase" in respect of the apparent specificity of the enzymes for the respective amines (Zeller 1938).

Complications arose with respect to this classification as it was shown that monoamine oxidase could in fact oxidise long chain aliphatic diamines whereas diamine oxidase did not (Blaschko & Duthie, 1945) and in conjunction with the effect of carbonyl reagents which were shown to inhibit some of the amine oxidases Blaschko divided the enzymes into two distinct classes, outlined as follows (Blaschko, 1959):

Amine oxidases that are not affected by carbonyl reagents. Included in this group are the monoamine oxidases described by Zeller and a number of FAD-dependent enzymes including mouse liver histaminase and the enzyme isolated from the cytosolic portion of rat liver which deaminates the secondary amine groups of the polyamines, spermine and spermidine.

Amine oxidases that are susceptible to carbonyl reagents, for instance semicarbazide and hydroxylamine. This group includes the classical histaminase and the amine oxidases derived from plants, bacteria, plasma and rabbit liver. Also included are ruminant plasma-borne polyamine oxidases that deaminate the polyamines spermine and spermidine at the primary amino group (Morgan, 1985) and benzylamine oxidase (Blaschko *et al.*, 1959).

In 1957 a soluble enzyme in horse serum which rapidly oxidised the artificial substrate benzylamine was discovered (Bergeret, 1957) and named benzylamine oxidase, not only based on the name of the substrate it preferentially oxidised but as Blaschko stated in 1962 "because benzylamine was rapidly oxidised, and because it became apparent at once that the properties of the horse plasma enzyme differed fundamentally from those of the intracellular amine oxidase" (see Lewinsohn, 1984). This enzyme would fit into the second group mentioned previously because it was inhibited by semicarbazide, whereas it did not inhibit enzymes from the first group. This group of enzymes have now been renamed the semicarbazide-sensitive amine oxidases. Included within this second group are enzymes such as histaminase, diamine oxidase, benzylamine oxidase and also lysyl oxidase, which is involved in the production of collagen (Siegel & Fu, 1976) along with cross-linkage formation (Kagan, 1986).

1.1.2 Modern Nomenclature of amine oxidases.

For convenience the modern nomenclature of amine oxidases as taken from the database belonging to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) <http://www.chem.qmul.ac.uk/iubmb/enzyme/> is described:

EC 1.4.3.4

Common Name : amine oxidase (flavin-containing)

Reaction: $RCH_2NH_2 + H_2O + O_2 = RCHO\ NH_3 + H_2O_2$

Other name(s): monoamine oxidase; tyramine oxidase; tryaminase; amine oxidase; adrenalin oxidase; epinephrine oxidase; MAO; polyamine oxidase; serotonin deaminase; adrenaline oxidase; spermidine oxidase; spermine oxidase; monoamine O_2 oxidoreductase (deaminating)

Systematic name: amine:oxygen oxidoreductase (deaminating) (flavin-containing)

Comments: A flavoprotein (FAD). Acts on primary amines, and usually also on some secondary and tertiary amines.

EC 1.4.3.6

Common name: amine oxidase (copper-containing)

Reaction: $RCH_2NH_2 + H_2O + O_2 = RCHO\ NH_3 + H_2O_2$

Other name(s): diamine oxidase; diamino oxhydrase; histaminase; amine oxidase; monoamine oxidase; amine oxidase (pyridoxal containing); benzylamine oxidase; histamine deaminase; histamine oxidase; Cu-amine oxidase; amine oxygen

oxidoreductase; diamine :O_2 oxidoreductase (deaminating); semicarbazide-sensitive amine oxidase; vascular adhesion protein-1

Systematic name: amine :oxygen oxidoreductase (deaminating) (copper-containing)

Comments: A group of enzymes including those oxidising primary monoamines, diamines and histamine. They are copper quinoproteins.

1.2 Monoamine Oxidases (MAO)

The monoamine oxidase "branch" of the amine oxidase family has by now been well studied and characterised although new information regarding its physiological role is constantly produced.

The enzyme itself belongs to the EC class 1.4.3.4 and is a flavin adenine dinucleotide (FAD) containing molecule (Tipton, 1967) The structure of FAD can be seen in Fig. 1.1.

This molecule is derived from riboflavin (vitamin B_2) which is enzymatically converted to flavin mononucleotide and subsequently FAD (Figure. 1.1). Fig 1.2 shows the main source of FAD located within the B vitamin. As the co-factor, this molecule is bound at the active site via a cysteine group (Cys-397) which is essential for activity (Wu *et al.*, 1993) along with Glu-34 and Tyr-44 (Zhou *et al.*, 1995a and 1995b) and is responsible for oxidising the substrate. Both MAO A and B have one FAD molecule per subunit (Weyler, 1989).

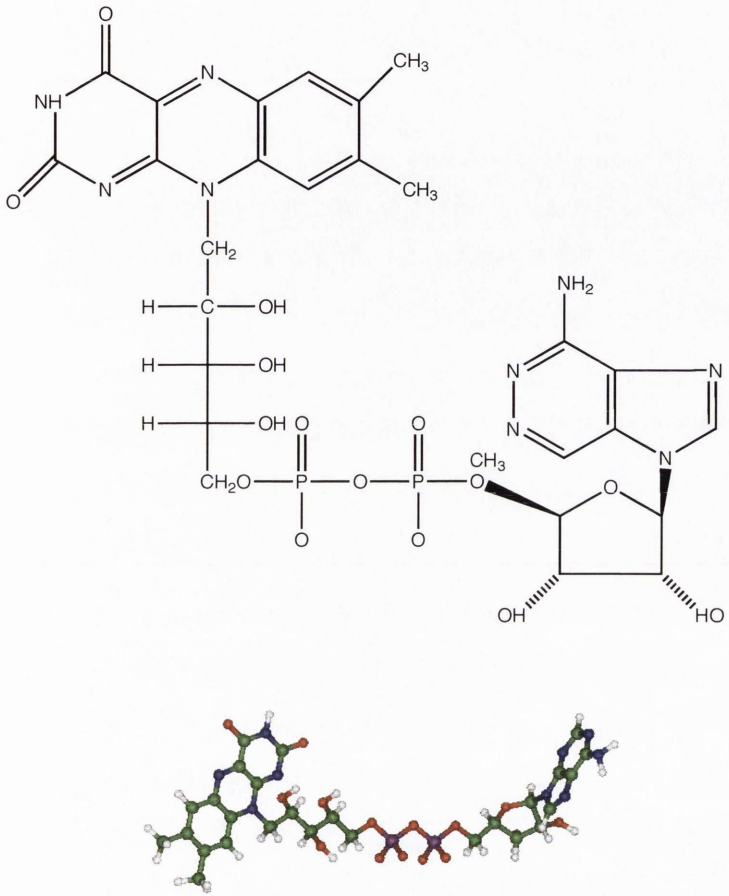


Fig. 1.1. The chemical structure and a 3-D model the co-factor of Flavin Adenine Dinucleotide (FAD) present within monoamine oxidase.

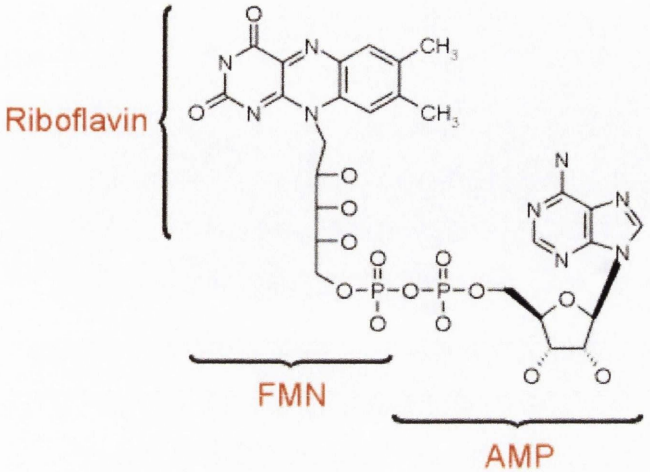


Fig. 1.2. Primary source of FAD; positioned within the vitamin B_2

MAOs have diverse molecular weights depending on the location, source and isoforms of the enzyme, e.g., 65 KDa (human Liver MAO B) (Lu *et al.*, 1996), 89 KDa (bovine brain MAO B) (Aoki *et al.*, 1977) and can exist as a functionally active monomer or a homo dimer. Each subunit contains a covalently bound flavin and is as functionally and similarly active as the monomer. A 3-D structure of a monomer unit of MAO is shown in Figure. 1.3.

Fig. 1.3. Three-Dimensional structure of human monoamine oxidase B, shown as a hetero-dimer, along with the FAD molecules located within each subunit (A) (Binda *et al.*, 2002). For comparison purposes the structure of another flavin containing amine oxidase , Polyamine oxidase is shown (Binda *et al.*, 1999) Colours are used to discrimainated between the various structural features present.

A

B

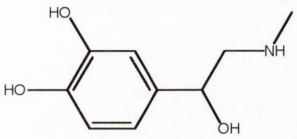
Both isoforms of the enzyme, MAO-A and MAO-B, are located on the mitochondrial outer membrane (Yoo *et al.*, 1976), along with a smaller proportion within the microsomal fraction. Immunohistochemical staining shows incorporation of MAO in the endoplasmic reticulum (Pekkel *et al.*, 1985) and both enzymes are found present in most mammalian and avian tissues at various levels (see table 1). They have the ability to oxidise aliphatic and aromatic primary amines along with some secondary and tertiary amines and there is a significant overlap in the substrate specificities of the two isoforms, although there are some amine substrates which are specific to one or other of the isoforms (see Figure 1.4). Differences in substrate specificity may be attributed to differences between microenvironment at the binding sites of the enzymes, MAO A appeared to be heterogeneous while MAO B homogenous (Huang & Eiduson, 1977). Specificity may also be determined by the enzymes location within a cell (Waldmeier, 1987) and as shown by using deuterated tyramine stereo-specificity can also play a role (Yu *et al* 1986).

Relative Activity	**MAO A**	**MAO B**
Very High	Placenta, Liver, Brain, Endometrium, Kidney	Liver, Brain, Lung, Nerve tissue, Glandular tissue
High	Smooth muscle, heart, Lung, Adrenal gland	Platelet, heart, Adrenal gland
Moderate	Vascular tissue, Skeletal muscle	Myometrium, Skeletal muscle, Vas deferens
Low	Spleen, Skin, Vas deferens	Spleen, Skin, Smooth muscle, Blood vessels

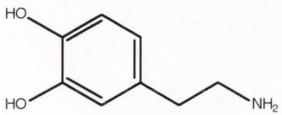
Table 1. Comparison of MAO A and MAO B relative activities in some tissue types, modified from Lewinsohn 1984.

Fig. 1.4. Common monoamine oxidase substrates, arranged according to their preferences for MAO-A, MAO-B or both.

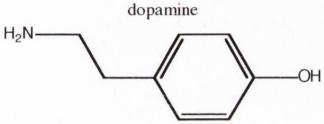
Common



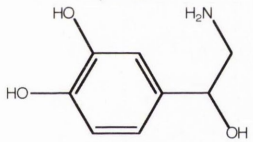
adrenaline



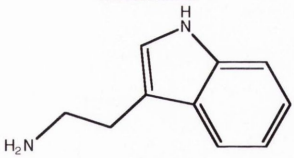
dopamine



tyramine

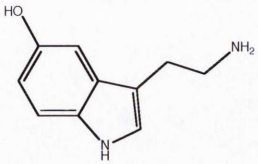


noradrenaline



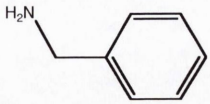
tryptamine

MAO-A

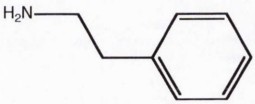


5-Hydroxytryptamine

MAO-B



benzylamine



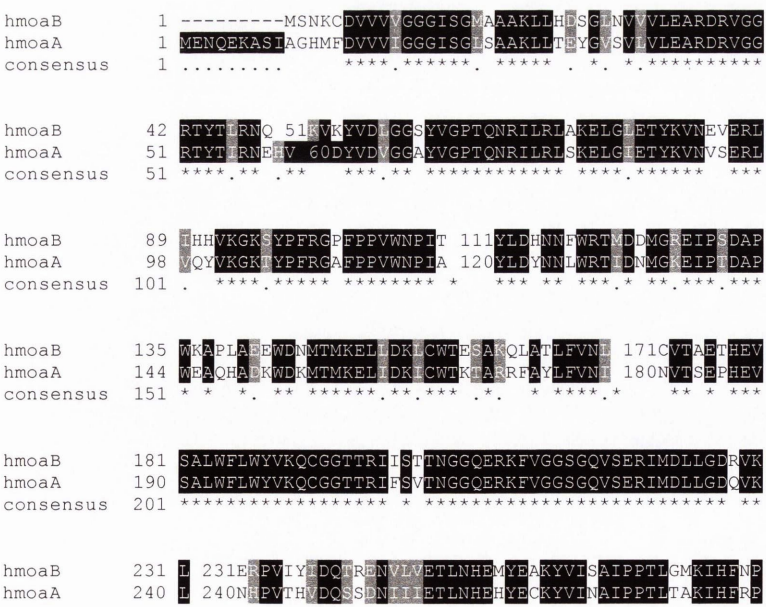
2-phenylethylamine

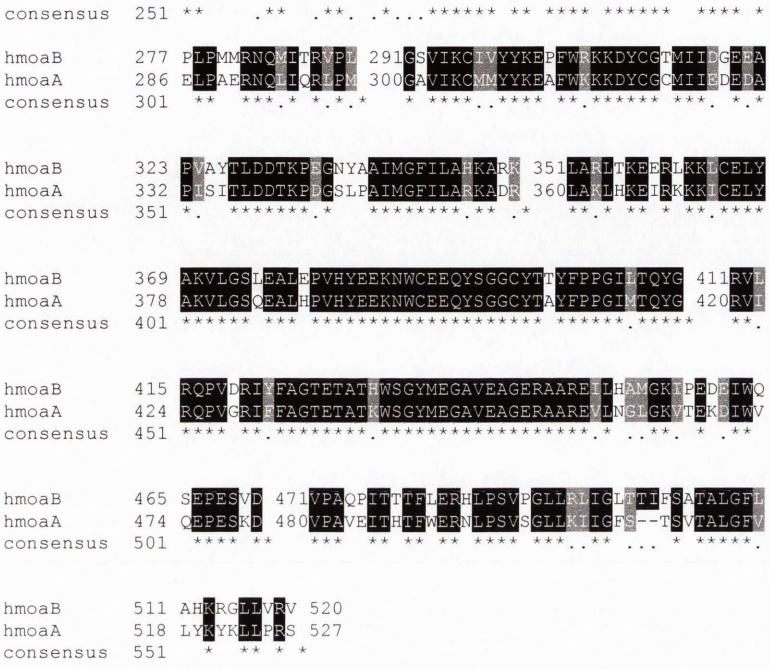
1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine
(MPTP)

1.2.1 Sequence analysis of MAO

Sequence analysis of both isoforms of monoamine oxidase, MAO A and MAO B unsurprisingly show substantial homology with one another. However one aspect which is surprising is that the homology is greater between the isoenzymes from differing species than between the two enzymes from the same source. A sequence identity of approximately 70% exists between the two enzymes from ox while there is a 90% similarity between human MAO B and ox MAO B. Similarly rat MAO A showed greater than 85% homology with human and bovine MAO A while a 70% homology with rat MAO B (Kwan *et al.*, 1992). Below is a boxshade representation of the two human isoenzymes MAO A and MAO B, which shows the (70%) similarity in sequence homology.

Sequence analysis was performed as described in section 1.3.9.




consensus 251
hmoaB 277 PLPMMRNQMITRVPL 291GSVIKCIVYYKEPFWRKKDYCGTMIIDGEEA
hmoaA 286 ELPAERNQLIQRLPM 300GAVIKCMMYYKEAFWKKKDYCGCMIIEDEDA
consensus 301
hmoaB 323 PVAYTLDDTKPEGNYAAIMGFILAHKARK 351LARLTKEERLKKLCELY
hmoaA 332 PISITLDDTKPDGSLPAIMGFILARKADR 360LAKLHKETRKKKICELY
consensus 351
hmoaB 369 AKVLGSLEALEPVHYEEKNWCEEQYSGGCYTTYFPPGILTQYG 411RVL
hmoaA 378 AKVLGSQEALHPVHYEEKNWCEEQYSGGCYTAYFPPGIMTQYG 420RVI
consensus 401
hmoaB 415 RQPVDRIYFAGTETATHWSGYMEGAVEAGERAAREILHAMGKIPEDEIWQ
hmoaA 424 RQPVCRIFFAGTETATKWSGYMEGAVEAGERAAREVLNGLGKVTEKDIWV
consensus 451
hmoaB 465 SEPESVD 471VPAQPITTTFLERHLPSVPGLLRIIGLTTIFSATALGFL
hmoaA 474 QEPESKD 480VPAVEITHTFWERNLPSVSGLLKIIGFS--TSVTALGFV
consensus 501
hmoaB 511 AHKRGLLVRV 520
hmoaA 518 LYKYKLLPRS 527
consensus 551

1.2.2 The genetics of monoamine oxidase

The location of the gene responsible for the production of MAO A was found situated on the X chromosome in humans (Pintar *et al.*, 1981). Further work revealed the chromosomal location to be Xp21-p11 (Ozelius *et al.*, 1988). MAO B has also been mapped to the X chromosome with suggestions that both these X-linked genes are structural genes for the enzymes (Kochersperger *et al.*, 1986). Both enzymes are now believed to be distinct gene products although the genes are very close to each other on the X chromosome and to the DXS7 locus (Xp 11.3) (Lan *et al.*, 1989). The actual gene structure for MAO A and B comprises of 15 exons (interspersed by 14 introns), spanning at least 60 kb and exhibit identical exon-intron organisation. These findings suggest that the MAOA and MAOB genes are derived from the duplication of a common ancestral gene (Shih *et al.*, 1993) The core promoter region of MAO A is comprised of two 90 bp repeats each of which contains two Sp1 elements and lacks a TATA box. The MAO B core promoter region contains two sets of overlapping Sp1 sites, which flank a CACCC element all upstream of a TATA box (Shih *et al.*, 1994). This difference in the promoter regions of the two genes may explain, at least in part, the different cell and tissue expression of the enzymes.

Several controlling factors regulating the expression of MAO genes have been identified, Sp1 was found to be a transcription factor for MAO A (Zhu *et al.*, 1994). Sp1 also has control over the expression of MAO B along with Sp4 by trans-activation of the gene through the proximal cluster of Sp1 sites and this activation can be repressed by over expression of Sp3 and a related family member BTEB2 (Wong *et al.*, 2001). Dexamethasone causes selective expression of MAO B over MAO A in cultured rat astrocytes (Carlo *et al.*, 1996). MAO B is also believed to undergo selective induction by the activation of protein kinase C and MAPK signalling pathway and c-Jun and Egr-1 appear to be the targets of this regulation (Wong *et al.*, 2002).

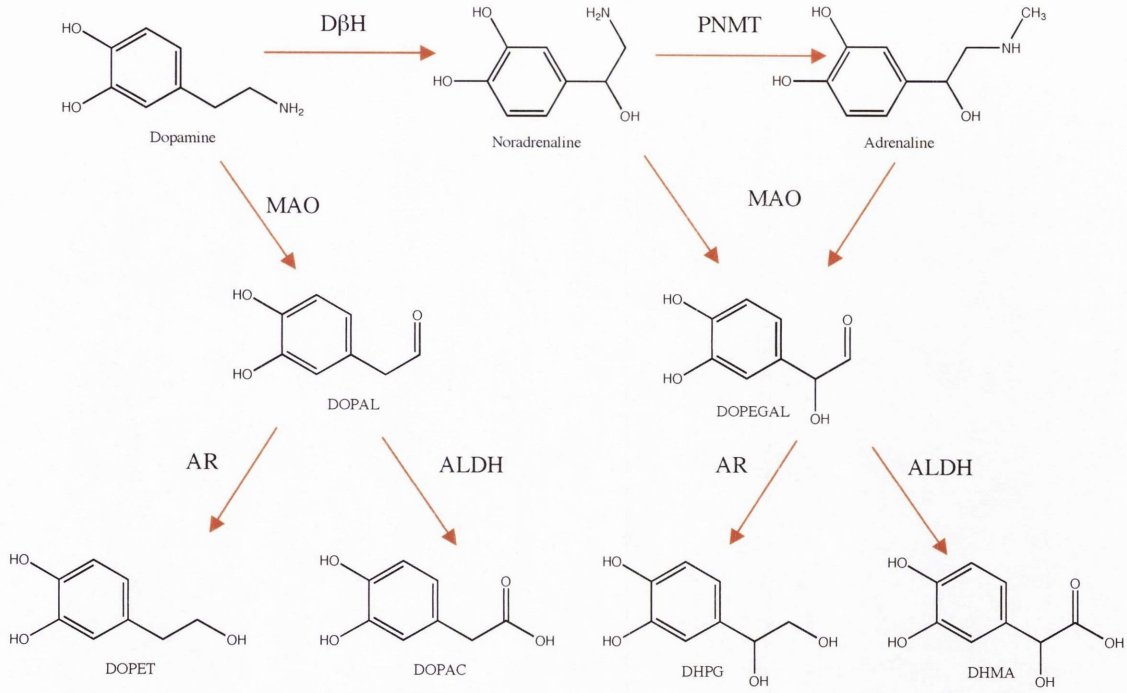
1.2.3. Primary function of MAO

Both MAO A and MAO B are involved in the metabolic degradation and regulation of endogenous amine substrates such as the neurotransmitters 5-hydroxytrptamine (5-HT, serotonin) by MAO A and 2-phenylethylamine (PEA) by MAO B. Dietary amines and other substrates, such as those mentioned in figure 1.4, are freely oxidised by MAO, albeit to lesser extent or specificity than those already mentioned. Both enzymes also play an important role in the metabolism of some xenobiotics.

Both MAO A and MAO B catalyse the oxidation of amines according to the overall reaction:

As can be seen from the reaction above the removal of amines, some of which can be neuroactive and vasoactive (von Korff,1977), leads to the production of ammonia (NH_3) or an amine, hydrogen peroxide (H_2O_2) and the corresponding aldehyde, each of which are potentially toxic. The aldehydes of course can be further metabolised to either carboxylic acids or alcohols by aldehyde dehydrogenase or aldehyde reductase, respectively. In the presence of iron (II), hydrogen peroxide can be converted to a highly toxic hydroxyl radical (•OH) by the Fenton reaction. This suggests an increase in amine metabolism may instigate oxidative injury to a cell (Wei *et al.*, 1996). A partial picture of the role MAO plays can be seen in figure 1.5 where an outline of some pathways of catecholamine metabolism.

Fig. 1.5. Pathways of catecholamine metabolism in which MAO is involved. DβH, dopamine β-hydroxylase; PNMT, phenolethanolamine-*N*-methylatransferase; AR, aldose or aldehyde reductase; ALDH aldehyde dehydrogenase; MAO, monoamine oxidase. DOPAL, 3,4-dihydroxyphenylacetaldhyde; DOPEGAL, 3,4-dihydroxyphenylglycoacetaldhyde; DOPET, 3,4-dihydroxyphenylethanol; DOPAC, 3,4-dihydroxyphenylacetic acid; DHPG, 3,4-dihydroxyphenylglycol; DHMA, 3,4-dihydroxymandelic acid.


HO
HO
NH2
Dopamine
DβH
HO
HO
H2N
OH
Noradrenaline
PNMT
HO
HO
CH3
NH
OH
Adrenaline
MAO
MAO
HO
HO
O
DOPAL
HO
HO
O
OH
DOPEGAL
AR
ALDH
AR
ALDH
HO
HO
OH
DOPET
HO
HO
O
OH
DOPAC
HO
HO
OH
OH
DHPG
HO
HO
O
OH
OH
DHMA

1.2.4. MAO pathology

As with all proteins a disruption in their function, whether it be due to up regulation, down regulation or absence (of a particular protein or enzyme), will inevitably lead to physiological complications. As can be seen Fig. 1.5 a disorder within MAO pathways will have substantial effects on the levels of physiological amines, e.g. neurotransmitters. What follows is a brief description of several disorders associated with MAO dysfunction

- MAO and Norrie's disease

Norrie's disease also known as retinal dysplasia is an X-linked syndrome resulting in retinal malfunction combined with mental retardation and deafness (Holmes 1971). Blindness usually presents at birth or during the first few months of life. Dementia or psychosis is present in 25% of cases and deafness in 20% of cases (Warburg 1971). Genetic markers for Norrie's disease were located on the proximal short arm of the X-chromosome (Bleeker-Wagemakers *et al.*, 1985) and the cause of the disorder has been attributed to a gene deletion in this area, which has allowed carrier detection (de la Chapelle et *al.* 1985). The relationship between MAO and Norrie's was elucidated through comparison of MAO levels and expression (both undetectable in patient fibroblasts and platelets) in two males whose family suffers from a sub-microscopic deletion resulting in Norrie's disease (Sims *et al.*, 1989). Since this relationship arose, deletions of both MAO A and MAO B have been implicated in atypical Norrie's disease (Lan *et al.*, 1989) (Levy *et al.*, 1989) along with changes in amine concentrations (Murphy *et al.*, 1990). A gene for Norrie's disease has been found distinct from the MAO genes but located adjacent to the MAO gene (Sims *et al.*, 1989). The gene arrangement for MAO genes and the Norrie disease gene (NDP) located in the p11.4-p11.3 on the X chromosome is tel-MAO A-MAO B-NDP-cent and is conserved in the mouse as tel-MAO B-MAO A-NDP-cent (Chen *et al.*, 1995). The discovery of a separate gene for Norrie's disease has led to the acknowledgement that the MAO genes alone are not responsible and the generation of differing phenotypes for the disease but extensive

deletions involving a NDP, MAO A and MAO B (and some additional material) phenotype result in a more severe disease state (Suarez-Merino *et al.*, 2001).

- Criminality

There appears to be a relationship between MAO levels and criminal behaviour. A correlation was shown between low platelet MAO levels in diagnosed psychopaths when compared to a control group (Lidberg *et al.*, 1985). Further studies have showed that, in former delinquents, MAO levels returned to that of control groups although they had previously reported to have low levels (Alm *et al.*, 1994). Genetic characterisation of a family where several of the male family members exhibited antisocial behaviour, such as impulsive aggression, arson and attempted rape, showed a point mutation in exon 8 of the MAO A gene which resulted in lower MAO A activity in these individuals (Parsian, 1999). This correlation is not exclusive to males and studies have shown antisocial females to which exhibit far lower MAO levels than the control groups (af Klinteberg, 1996). More recent reports continue to suggest the role of MAO in criminal behaviour (Stalenheim 2001) and (Garpenstrand *et al.*, 2002) so the possibility should not be ignored.

- Alcohol, cigarettes and drug dependence

Substance abuse is a chronic problem in today's modern world costing governments countless millions in healthcare, rehabilitation and prevention, not to mention the cost of the associated criminal aspects. Many studies have since been dedicated to try and find associations between biochemical and genetic markers. Many such studies have focused on the relationship between MAO and addiction of alcohol, cigarettes and other abuse substances, such as cocaine and opium. Initial studies showed a pattern of low platelet MAO in alcoholics (Takahashi *et al.*, 1976) and chronic marijuana users (Stillman *et al.*, 1978). This was reversed with prolonged withdrawal from alcohol (Wiberg. 1979), although the activity of the enzyme can remain unstable during the period of withdrawal

(Alexopoulos *et al.*, 1981). The low level of platelet MAO in alcoholics has been substantiated in numerous reports and has also been extrapolated to the relatives of alcoholics (Alexopoulos *et al.*, 1983). Similarly tobacco smokers were reported to have low platelet MAO A and B levels (Berlin *et al.*, 1995) and to be prone to further addictions (Von Knorring & Oreland 1985 A). However as these subjects were already smokers a low level of MAO would be expected due to inhibition of MAO by some components of tobacco smoke (Fowler *et al.*, 1996 a & b) as opposed to a biological characteristic of smokers (Fowler *et al.*, 2003) As progress is made in this area of study, further distinctions can be made relating to platelet MAO and alcohol abuse by using MAO levels in distinguishing between the two types of alcoholism, Type I and Type II. Type II is the more severe of the two types and it is this form that is now associated with low platelet MAO, whereas Type I alcoholic levels were comparable to healthy controls in an early study (Von Knorring *et al.*, 1985 B). More recent studies have showed that, while Type II alcoholics have a lower MAO level than Type I, Type I does show significantly lower MAO activity than the normal controls (Von Knorring *et al.*, 1991). Dependence on class A drugs such as cocaine and heroin shows a correlation with the presence of high platelet MAO activity in users, possibly due to regulation by the drug (cocaine is an amine) (Macedo *et al.*, 1995) and results in an increase in extra-cellular dopamine and noradrenaline levels. This effect caused by cocaine does not respond to treatment with MAO A or MAO B inhibitors (Pepper *et al.*, 2001).

- Attention Deficit Disorder (ADD)

Attention Deficit Disorder is a syndrome, usually diagnosed in childhood, characterised by a persistent pattern of impulsiveness, a short attention span and often hyperactivity which can interfere with academic, occupational and a child's social performance. Several studies have resulted in seemingly contradictory information regarding the levels of MAO in sufferers of ADD, i.e. some report reduced levels of MAO in ADD (Shekim *et al.*, 1982 and 1986), while others suggest that MAO is raised in similar disruptive behaviour disorders (Stoff *et al.*, 1989). What is confirmed is that there is a genetic link between such disorders and the DXS7 locus on the X chromosome which is closely

associated with the MAO genes (Jiang *et al.*, 2000; Jiang *et al.*, 2001). The effectiveness of MAO inhibitors (MAOIs) in reducing or controlling patient symptoms is also compelling evidence to establish a connection between MAO and ADD (Boix *et al.*, 1998; Wender 1998; Vaiva *et al.*, 2002).

- Panic Disorder, Social Phobias and Depression

Common symptoms of psychiatric illness are usually acute anxiety or panic disorder, which can manifest as discrete episodes of severe acute anxiety and possible agoraphobia or claustrophobia. In studies of patients which exhibited such symptoms, MAO inhibitors have reportedly relieved symptoms (Boulenger & Uhde 1983). There have also been reports of elevated platelet MAO in such patients, in comparison with normal control subjects (Gorman *et al.*, 1985). Most subsequent research has focused on the effect of MAOIs reducing symptoms and the effect on MAO activity rather than elucidating any correlation between MAO levels and the disorder (van Vliet *et al.*, 1996). With the use of MAOIs in the treatment of panic disorder interest has focused on analysis of the MAO gene in order to determine a possible cause and to show, in certain groups, the presence of longer alleles (which were more active) in the promoter region of the MAO gene. Such were the findings when significantly more frequent occurrences of the longer alleles were found in female patients than controls (Deckert *et al.*, 1999), this is now considered a risk factor for such disorders in females (Schulze *et al.*, 2000). A similar situation exists in the case of patients with social phobias such as agoraphobia (Yu *et al.*, 1983 a & b) and treatment with MAOIs has proved to be effective in the relief of social phobias (Den Boer *et al.*, 1995).

Research into causes of depression and the role of neurotransmitters (Lapierre 1982) and monoamines (Strolin-Benedetti 1982) has been far more prevalent than with the, previously mentioned, social disorders and brings with it a deluge of reports describing the role of MAO and the use of MAOIs within depression (Feldstein *et al.*, 1965). Initial work suggests an increase in MAO activity is at least partially responsible (Sandler *et al.*, 1975). No significant difference was found when MAO levels were determined in depressed patients albeit using serotonin, which is only a MAO A substrate (Takahashi

1977). Further studies substantiate this, and suggest that within subgroups of bipolar patients there could be differences in the levels of platelet MAO (Edwards *et al.*, 1978), and this was confirmed in studies of the differing subgroups (Orsulak *et al.*, 1978). For treatment of depression with MAOIs to be effective, at least 60% reduction in MAO activity has to be achieved, as shown in the case of phenelzine administration (Davidsion *et al.*, 1978). Since the advent of treating depression using MAOIs, most work has involved the development of new more specific inhibitors, such as moclobemide, which is a reversible MAO A inhibitor (Stefanis *et al.*, 1988), and then to determine the clinical and potency of such drugs, as well as any side effects (Casacchia & Rossi 1989) and benefit over the older MAOIs (Lecrubier 1994). Studies have focused on the differing isoforms of the enzyme, which have shown if inhibition of one over the other results in any significant effects on the treatment of depression (Sofuoglu *et al.*, 1992). There are now many up to date reviews which discuss in greater detail the role of MAO in depression and the use of MAOIs as a treatment, see (Wouters 1998; Baker *et al.*, 1999; Gareri *et al.*, 2000; Riederer *et al.* 2004)

- Neurodegeneration

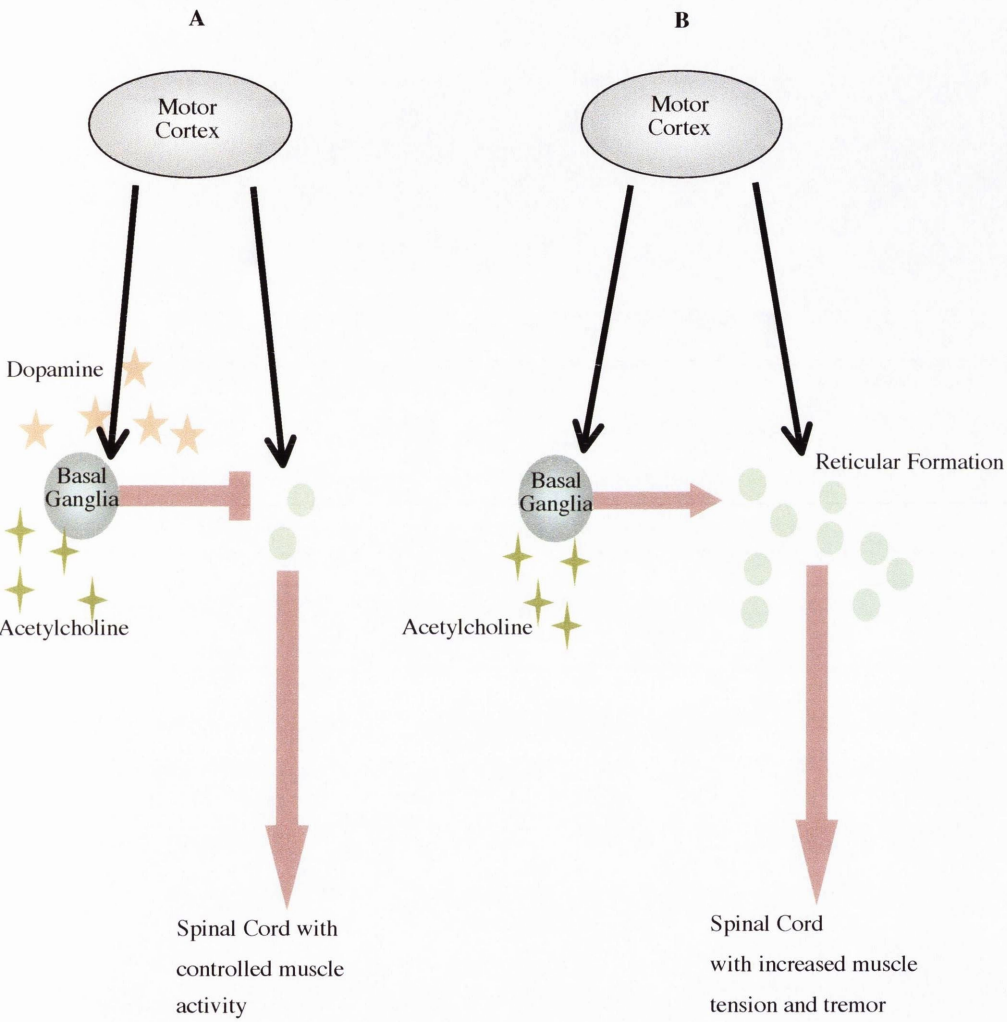
Two of the most prevalent neurodegenerative disease states today are Parkinson's and Alzheimer's disease. Parkinson's disease (PD) was first noticed by a British physician James Parkinson who, in 1817, observed on the streets of London that some people suffered from tremors and muscle weakness which over a period of time worsened, Parkinson published these observations in a essay titled "Essay on the Shaking Palsy" with the result that his name became associated with the disease to this day (Parkinson, 1817). The cause of PD is still not known but there are several forms of PD in which other disease states such as influenza, encephalitis, herpes simplex virus (HSV) and HIV seem to have a role to play. Several case studies report the onset of PD after patients have suffered from encephalitis, (see Schultz *et al.*, 1977). A probable hypothesis for the appearance of viral antibodies such as HSV within PD is due, in part, to the general disturbance of such patients' immune functions (Marttila *et al.*, 1982).

Parkinson's Disease is a disease of the basal ganglia; the major characteristic is the progressive loss of dopaminergic neurons within the substantia nigra thereby resulting in the loss of dopamine (DA), which is a major neurotransmitter involved in motor function. This idea was first proposed by Carlsson in 1958 and confirmed in his paper the following year (Carlsson, 1959). This work was then substantiated a year later (Ehringer & Hornykiewicz 1960) and has since been the primary model used to describe PD. A schematic representation of the role dopamine plays in PD is shown in Fig 1.6. The reduction in DA is due to a degeneration of the dopamine cells within the substantia nigra. Although dopamine loss is the earliest and most marked neurochemical aspect of the disease there are also depletions in noradrenaline and acetylcholine.

As MAO is essential for the degradation of dopamine a link between the enzyme and PD would appear reasonable. L-dopa is given to PD sufferers as a method of replacing dopamine and has the added effect of reducing MAO levels, thereby preventing the oxidation of the replaced dopamine (Zeller *et al.*, 1976). *l*-Deprenyl (selegiline), a specific inhibitor of MAO B also relieves some of the symptoms associated with PD, albeit with limited success, and side effects such as insomnia (Eisler *et al.*, 1981). Deprenyl treatment is dependent on the state of the disease at the time of administration (Portin & Rinne 1983) and appears to have advantages over the traditional L-dopa treatment regime including no noticeable side effects (Ruggieri *et al.*, 1986). An obvious path to follow was to use combination treatment that allowed the reduction of the L-dopa dose yet still allowed for a relief of symptoms. However in order for this regime to be effective treatment needs to commence in the early stages of the disease (Rinne 1989). Although deprenyl is known to inhibit MAO B activity in PD and therefore reduce the oxidation of dopamine there is also speculation that it may also up-regulate anti-oxidant and anti-apoptotic molecules, which help prevent the cell death of DA neurons (Olanow 1996). Prevention of apoptosis in retinal cells by deprenyl (Xu *et al.*, 1999) and inhibition of MPP^+ induced apoptosis (Sharma *et al.*, 2003) was also observed. Confirmation of this role for deprenyl has coincided with the emergence of a host of other mechanisms, such as the up-regulation of superoxide dismutase and catalase, which help prevent formation of the oxygen radicals, involved in the disruption of the DA neurons (Ebadi *et al.*, 2002).

Genetic links are now beginning to surface relating MAO to PD including a link between a MAO A polymorphism and PD susceptibility (Nakatome *et al.*, 1998) along with a GT repeat polymorphism on the MAO B gene which may also be a link to PD susceptibility (Mellick *et al.*, 2000), but no definite link has been established and the consensus appears undecided warranting further research in this area.

Fig 1.6. A schematic representation of the role of the neurotransmitter dopamine in Parkinson's disease. As dopamine levels drop in patients with PD control over reticular formation is rescinded leading to increased muscle tension and tremor. (A) Graphical reperesentation of controlled muscle activity under normal circumstances compared to (B) uncontrolled muscle tremor resulting from increased reticular formation in the absence of dopamine


A
Motor Cortex
Dopamine
Basal Ganglia
Acetylcholine
Spinal Cord with controlled muscle activity
B
Motor Cortex
Basal Ganglia
Reticular Formation
Acetylcholine
Spinal Cord with increased muscle tension and tremor

A novel role for MAO in the occurrence of PD arose in the 1980s when, while attempting to formulate and manufacture a heroin analogue, drug addicts produced a compound contaminated with 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine (MPTP). MPTP is metabolised by MAO B to 1-methyl-4-phenylpyridinium (MPP^+), see Fig 1.7 (Chiba *et al.*, 1984; Singer *et al.*, 1984), a potent neurotoxin. MPP^+ enters the DA neurons via the dopamine transporter (Markey *et al.*, 1984) where it acts on the mitochondria by selectively inhibiting complex I of the electron transport chain with the resultant depletion of energy and production of free radicals (Scotcher *et al.*, 1990). For an, as yet unknown reason, MPP^+ appears to specifically accumulate in the DA areas within the substantia nigra but not within other DA areas of the brain such as the striatum (Langston 1985). The resulting MPP^+ DA depletion and the onset of PD symptoms occur within days, rather than the slow progression normally associated with the disease.

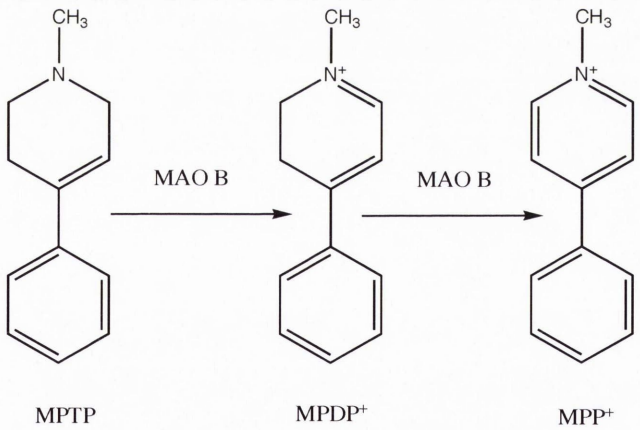


Fig. 1.7. The metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine (MPTP) to 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase B.

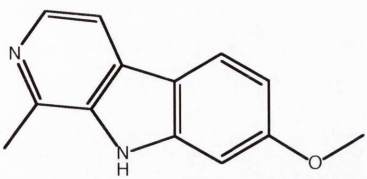
Alzheimer's disease (AD) first discovered in 1905 by the German physician Alois Alzheimer while treating a patient with symptoms described as "progressive cognitive impairment, speech and perception problems, hallucinations, delusions and social incompetence". An autopsy on the patient revealed abnormal plaques and neurofibrillary tangles of the brain, which are the hallmarks of the disease as recognised today. MAO, as a biochemical marker, is raised in patients suffering from Alzheimer-related dementia (Oreland & Gottfries 1986) along with an increase in MAO mRNA expression within disease associated brains (Emilsson *et al.*, 2002). An increase in MAO levels prompted the use of deprenyl as a treatment with some success as it alleviates some but not all of the cognitive functions affected by the disease (Tariot *et al.*, 1987). However a comprehensive analysis of studies undertaken using deprenyl to attenuated the effect of AD has since shown it to in ineffective for this purpose (Birks & Flicker, 2003). Studies looking at the possible involvement of DA neurons in AD have suggested that any decrease in DA neurons combined with increased MAO activity is due to coexistent PD (Reinikainen *et al.*, 1988). MAO activity has been associated with the senile plaques, where MAO B was found clustered within astrocytes in the plaques (Nakamura *et al.*, 1990). In a similar manner to PD, oxidative stress plays a substantial role in the progress of AD by free-radical induced damage to lipid and protein constituents of neuronal membranes. The action of MAO may, in part, contribute to the production of free radicals. This allows the use of antioxidants such as lazabemide, which is also a reversible MAO B inhibitor, to reduce damage associated with oxidative stress and reduce the production of free radicals by regulating the H_2O_2 produced by MAO B (Mason *et al.*, 2000). Research on AD is continuing on the development of novel MAOIs, which can confer increased specificity, potency and reduced side effects etc. (Youdim *et al.*, 2002).

1.2.5. MAO inhibitors

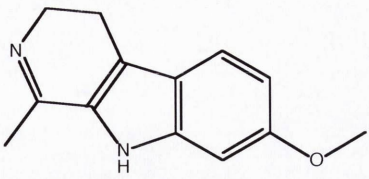
Monoamine oxidase inhibitors play a significant role in the pharmacology of many neurological disease states, as described in the previous section. The inhibition of the enzyme is linked to the co-factor FAD within the enzymes active site. Experiments with

the ^{14}C radiolabelled clorgyline and deprenyl (MAO A and MAO B inhibitors respectively) showed the formation of a covalent adduct between the inhibitor and FAD, which results in an irreversible inhibition (Salach *et al.*, 1979). Hydrazine compounds can also result in the irreversible inhibition of the enzyme along with cyclopropylamine, propargylamine and allylamine derivatives, (see Strolin Benedetti & Dostert 1987 for a review). The neurotoxin MPTP, although a substrate for MAO B, irreversible inhibition occurs as a competing process during the oxidation of MPTP by MAO-B, resulting in it behaving as a "suicide substrate" $MPDP^+$ is also a "suicide substrate" but not MPP^+ is a simple reversible inhibitor (Singer *et al.*, 1986; Tipton *et al.*, 1986). See section 4.3.1 for a more detailed discussion of MAO inhibition. Figure 1.8 lists some of the irreversible and reversible inhibitors of MAO.

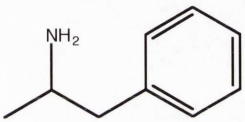
Fig. 1.8. Reversible (A) and irreversible (B) inhibitors of monoamine oxidase.



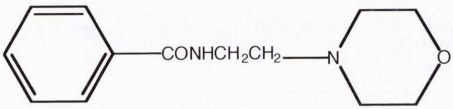
harmine



harmaline

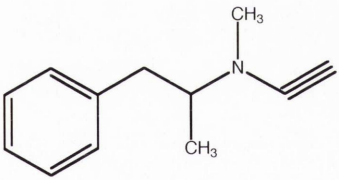


amphetamine

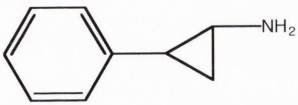


moclobemide

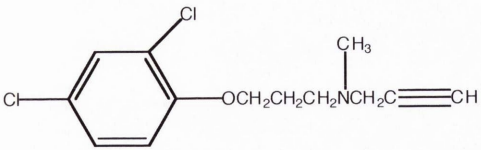
A



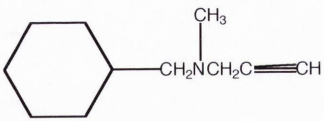
deprenyl (selegiline)



tranylcypromine



clorgyline



pargyline

B

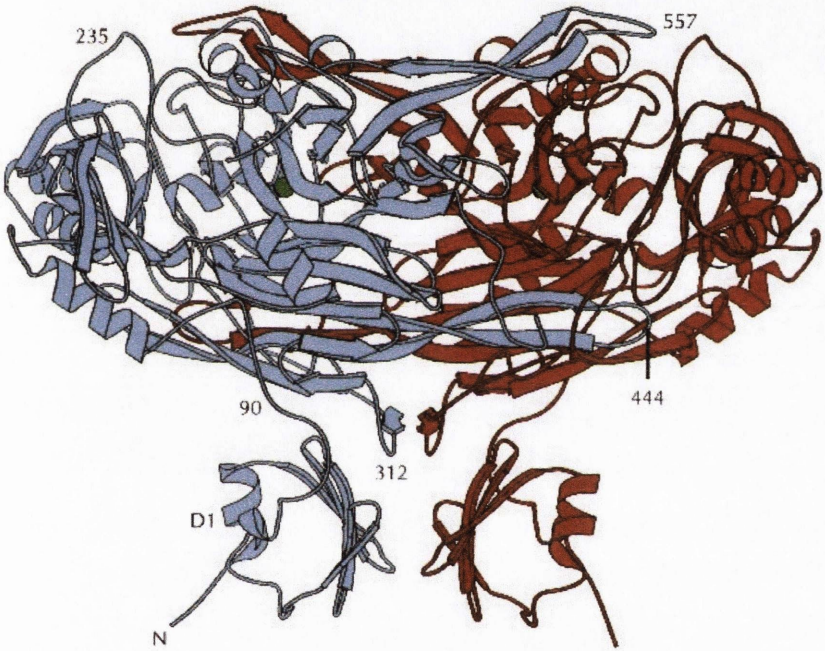
1.3. Semicarbazide Sensitive Amine Oxidase (SSAO)

Semicarbazide sensitive amine oxidases (SSAO) belong to the copper-containing group of amine oxidases (EC 1.4.3.6) and have been known by several names, such as benzylamine oxidase, due to its preference for benzylamine as a substrate, and clorgyline resistant amine oxidase, as it was not inhibited by clorgyline, unlike MAO. They are a ubiquitous group of enzymes found in plants, animals and microbes, (see Lewinsohn 1984; Callingham *et al.*, 1995; Lyles 1996 & Houen 1999). Most species contain a soluble form of SSAO in blood plasma plus a membrane bound form of the enzyme that has a relatively high activity in cardiovascular tissue, lung and adipocytes (see Boomsma *et al.* 2000; Lyles, 1995 & 1996).

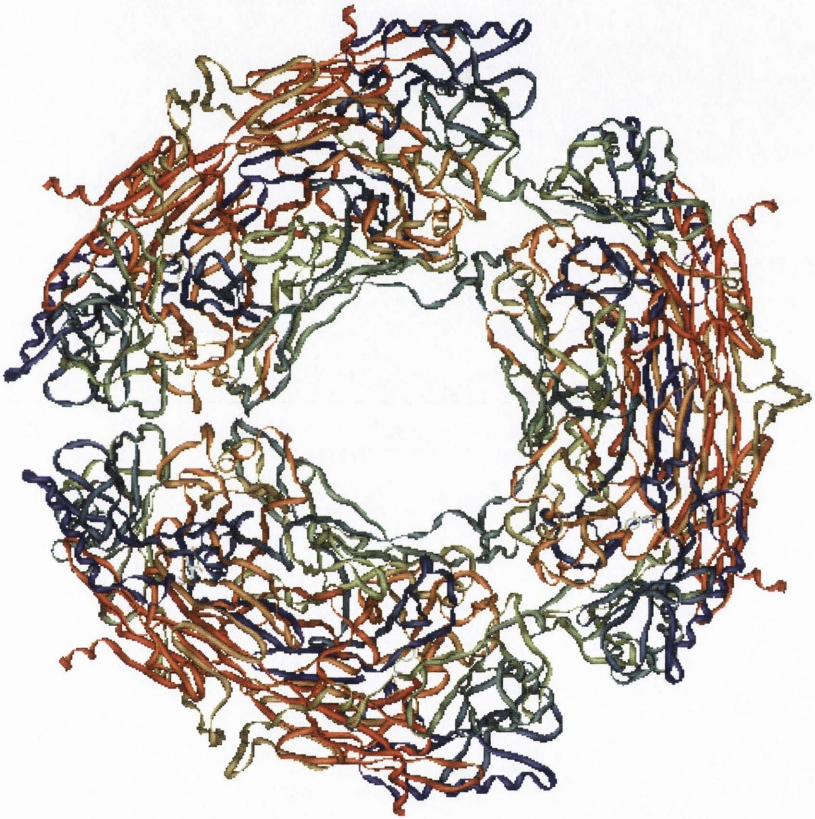
1.3.1 Structure of SSAO

SSAO exist in mammals usually as dimers (Yadav & Knowles, 1981) with an overall molecular weight of approximately 180,000 Da, with a subunit size of 90,000 Da and one atom of copper per subunit. (Buffoni & Blaschko 1964). Cloning of mRNA from several species has revealed a predicated amino acid sequence of 763 residues in length (Smith *et al.*, 1998; Moldes *et al.*, 1999) and heavily glycosylated (Bono *et al.*, 1999). A three-dimensional structure has recently been added to the protein data bank for the human form of the enzyme (hVAP-1) (Airenne *et al.*, 2003 unpublished) but crystal structures of several other copper containing amine oxidases have been available for some time and are shown in Fig. 1.9. A channel was identified which might allow transport of oxygen into the active site, this channel is formed at the interface of the two subunits and would appear to be solvent accessible (Parsons *et al.*, 1995). Although the structure of hVAP-1 is now known, the structure of human SSAO has yet to be clarified, using PERSCAN, made available by Dan Donnelly a prediction of the presence of alpha helices based on the amino acid sequence can be made (Donnelly *et al.*, 1994), see Fig. 1.10

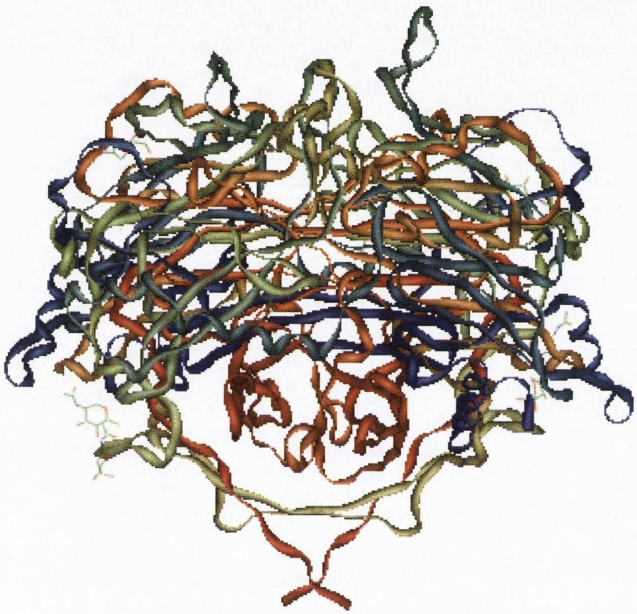
Fig. 1.9. Three-dimensional structures of five forms of semicarbazide sensitive amine oxidase. (A) *Escherichia coli* amine oxidase (Murray *et al.*, 1999) The two dimers are shown in blue and red, the copper atoms are represented in green. (B) *Hansenula polymorpha* amine oxidase (Li *et al.*, 1997). (C) Pea Seedling amine oxidase (Wilce *et al.*, 1996) and (D) VAP-1 (Airenne *et al.*, 2003 to be published). Colours are used to discrimainate between the various structural features present.



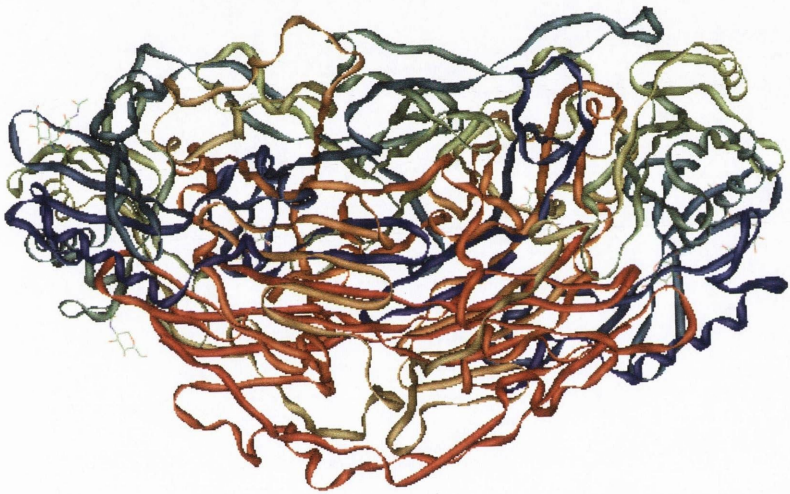
A



3



C

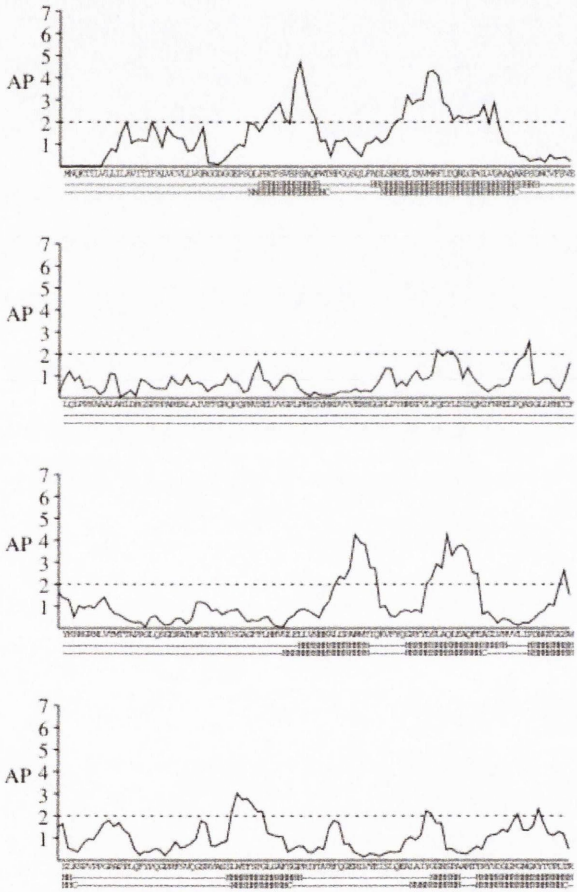


D

PERIODICITY IN HYDROPHOBIC RESIDUES

Example of big alignment

A AP Scan (window = 18)



Calculated using SCAN v7.0 - Dan Donnelly 1991,1992,1993

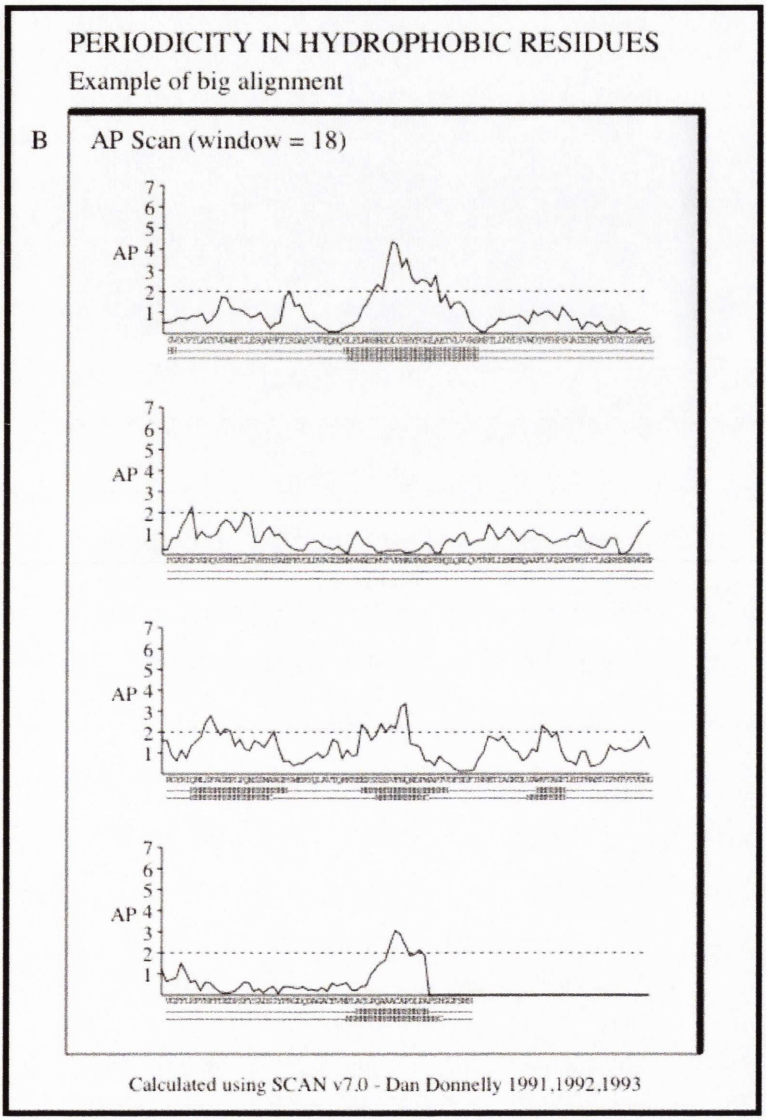


Fig. 1.10 Plot of alpha-helical periodicity derived from primary sequence of SSAO using the FORTRAN programme PERSCAN v7.0 by Dan Donnelly

1.3.2. Organic co-factors

Originally pyridoxal phosphate (PLP) was believed to be a co-factor for SSAO (Davison 1956). A reasonable assumption was that a carbonyl group must be involved due to the enzymes sensitivity to carbonyl reagents such as semicarbazide. PLP was purified as an adduct after acid hydrolysis of the pig enzyme (Buffoni 1990), but this may have resulted from contamination. Another contender as a possible co-factor was pyrroloquinoline quinone (PQQ), due to its involvement in bacterial redox systems (Duine & Frank 1981) but this was ruled out after a probable source of PQQ contamination was discovered (Buffoni *et al.* 1992). 6-hydroxydopa or 2,4,5-trihydroxyphenylalanine (TOPA), see Fig. 1.11 was then identified as the probable co-factor through MS and NMR analysis (Janes *et al.*, 1990) and further studies using resonance raman spectra were consistent with this (Brown *et al.*, 1991). Through isolation of the peptide apparently responsible for the TOPA quinone and comparison with the DNA sequence for the enzyme a specific codon, UAC, which codes for the amino acid tyrosine was found to correspond with the TOPA quinone in the mature protein (Mu *et al.*, 1992). The generation of the TPQ is a self-processing mechanism that utilises copper (II) and oxygen (Cai & Klinman 1994).

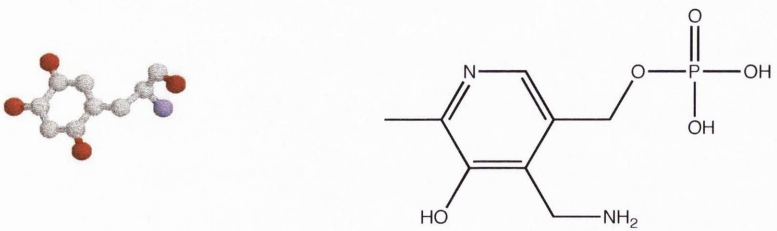


Fig. 1.11. Three-dimensional and chemical structure of the co-factor within semicarbazide sensitive amine oxidase (SSAO) 6-hydroxydopa; 2,4,5-trihydroxyphenylalanine (TOPA), which is a post-translational modification of a Tyrosine residue at the enzyme active site.

1.3.3. Reaction and mechanism

SSAO catalyses the same reaction as MAO, whereby an amine is converted to its aldehyde (RCHO) with the stoichiometric release of ammonia (NH_3) and hydrogen peroxide (H_2O_2). The reaction requires molecular oxygen and functions by a ping-pong mechanism (Kilnman 1996).

$$E\text{-}TPQ_{ox} + RCH_2NH_2 \longrightarrow E\text{-}TPQ_{red} + RCHO$$

$$E\text{-}TPQ_{red} + O_2 \longrightarrow E\text{-}TPQ_{ox} + NH_3 + H_2O_2$$

This is a two-step mechanism consisting of oxidative and reductive half reactions. As shown in the above scheme, the oxidised form of TOPA, TOPA quinone (TPQ) is reduced in the formation of the aldehyde and is then re-oxidised by molecular oxygen. Copper was assumed to play a significant role in the catalytic cycle of SSAO and in both the plasma and membrane bound forms of the enzyme. However it was established that it was not essential for the activity of rat brown adipose tissue SSAO (Barrand & Callingham 1984). The role of copper is now limited to the production of the TPQ co-factor as very little copper reduction has been shown in bovine SSAO and this would have insignificant effect on the catalytic mechanism (Dooley *et al.*, 1991), see section 4.2.2 for a description of the biogenesis of the TPQ co-factor. This has led to the conclusion that molecular oxygen in reduced SSAO does not bind the copper and the stereochemistry of O_2 is still unresolved (Bellelli *et al.*, 2000). However current descriptions of the mechanism now include copper as an intergral part of the overall reaction (Dove *et al.*, 2000). A detailed discription of the catalytic mechanism of SSAO is contained in section 4.2.2.

1.3.4 SSAO Substrates

There are large species differences in the specificities of SSAO with regard to substrates, but the non-physiological amine benzylamine is a good substrate for the mammalian enzymes. The physiological substrates are believed to include aminoacetone (Deng & Yu 1999) and methylamine (Yu 1998) (see Fig. 1.12), 2-phenylethylamine, tyramine (Young *et al.*, 1982) and dopamine (Lizcano *et al.*, 1991). MAO also oxidatively deaminates many of these SSAO substrates with the exception of aminoacetone and methylamine. SSAO also catalyses the oxidative deamination of a number of xenobiotics (Tipton & Strolin-Benedetti 2001).

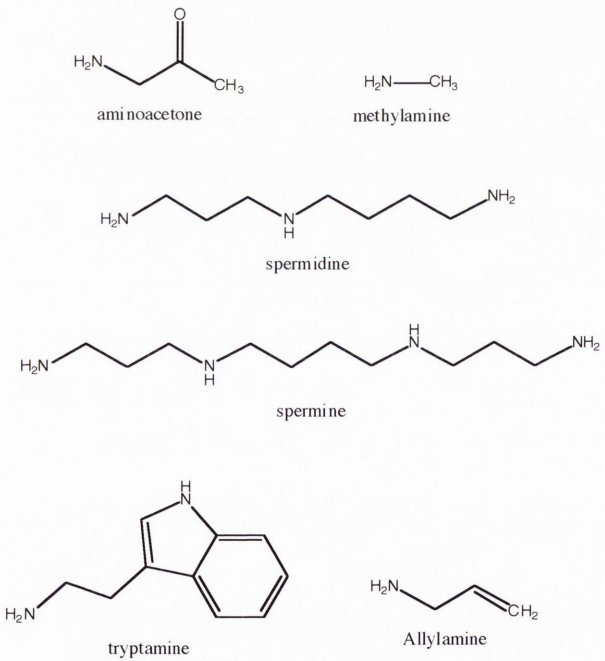
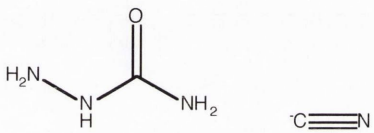


Fig. 1.12. Chemical structures of some SSAO substrates including the believed physiological substrates aminoacetone and methylamine.

1.3.5 SSAO Inhibitors

The most commonly used inhibitor of SSAO is the carbonyl reagent semicarbazide (Figure 1.13), which also lends its name to the enzyme and allows differentiation between SSAO and MAO, as both will cause the oxidative deamination of benzylamine but SSAO is inhibited by 0.1-1mM semicarbazide (Lewinsohn 1984). Other carbonyl reagents, such as cyanide, will also inhibit SSAO. The mechanism of SSAO inhibition is discussed in detail in section 4.3.3.



semicarbazide cyanide

Fig. 1.13. Chemical structure of the carbonyl reagents semicarbazide and cyanide.

MAO inhibitors, such as clorgyline and deprenyl, have a weak effect on SSAO but any effect is normally only seen at relatively high concentrations e.g. 1 mM concentrations of clorgyline cause poor inhibition of SSAO (Lewinsohn *et al.*, 1978). Hydrazine derivatives are among the most potent inhibitors of SSAO. These include procarbazine and methylhydrazine, although inhibition by procarbazine is believed to result from a metabolite of the compound (Holt & Callingham 1994). Phenylhydrazine is a potent irreversible inhibitor with an IC_{50} of 30 nM (Lizcano *et al.*, 1996). For a comprehensive review of the many compounds displaying inhibition of SSAO with varying degrees of potency and specificity see Lyles 1996.

1.3.6 Distribution of SSAO

The majority of SSAO is now known to be associated with cell membranes and blood vessels and appears to be a common cross species feature (Lewinsohn 1984). Vascular tissue was found to contain large amounts, as shown in rat aorta (Lyles & Singh 1985). SSAO has to date been located in kidney (Banchelli *et al.*, 1988), adipose tissue (Raimondi *et al.*, 1992), rat brain microvessels, meninges, retina and eye sclera (Zuo & Yu 1994). Plasma SSAO (pSSAO) appears to be the result of proteolytic cleavage of membrane bound SSAO and evidence suggests the primary source for the pSSAO is the liver (Kurkijärvi *et al.*, 1998). SSAO has also been isolated from microsomes in both large and small intestine, liver, stomach and lung in the rat (Ochiai *et al.*, 2005).

1.3.7 Pathology of SSAO

SSAO levels have been determined in various disease states in an attempt to correlate the enzymatic activity to the onset of disease. As a function of SSAO is scavenging amines, a point of interest is that, although the removal of some of these amines may be beneficial, in some cases the products obtained from their oxidation can be more toxic than the initial substrates. Metabolism of allylamine, a substrate for SSAO (Nelson & Boor 1982), gives rise to acrolein, which can induce vascular toxicity presenting as transmural scarring, ventricular aneurysm and metaplasia of the endocardial cartilage (Boor & Ferrans 1985; Boor & Hysmith 1987). Acrolein-related damage is prevented by semicarbazide (Ramos *et al.*, 1988), and is modulated by beta-aminopropionitrile (betaAPN) (Conklin *et al.*, 1999). Catalase reduced the extent of the damage caused by allylamine oxidation, which points toward a contributing role for H_2O_2 (Ramos *et al.*, 1988).

Aminoacetone oxidation also gives rise to a toxic aldehyde, methylglyoxal, while methylamine is oxidatively deaminated to formaldehyde. Excess SSAO related production of these compounds can give rise to endothelial injury, oxidative stress, plaque formation and comprise protein cross linking. Since formaldehyde aggravates advanced glycation, it has been proposed to be responsible for some of the complications

in diabetes, where the enzyme activity is increased (Yu 1998). Decreased activity is also seen in breast tumours (Lizcano *et al.*, 1991b) which was also described when a reduction in plasma SSAO activity was reported in patients with severe burns or solid tumours (Lewinsohn 1984), probably due to competition between the tumour and enzyme for essential elements such as copper (Elliot *et al.*, 1991).

1.3.8 Multiple functions of SSAO

- **Amine oxidation**

Although the physiological functions of SSAO are by no means fully understood, the primary role of SSAO is believed to be the scavenging of primary amines, either locally-released or circulating amines. The active site of the membrane bound form of the enzyme appears to be externally facing, thus allowing the metabolism of endogenous amines, such as methylamine, tryptamine and aminoacetone, as they circulate in the bloodstream. SSAO in concert with MAO facilitates the removal of tyramine in isolated perfused mesenteric arterial bed of the rat (Elliott *et al.*, 1989). The removal of dopamine may also be facilitated by SSAO, as shown in the rat vas deferens (Lizcano *et al.*, 1991). The oxidation of aminoacetone has been described in human umbilical artery (Lyles & Chambers 1992) and bovine lung (Lizcano *et al.*, 1994).

- **GLUT 4 translocation**

GLUT 4 is an isoform of the glucose transporter, the other main one being GLUT 1. GLUT 4 is located in adipose tissue, skeletal and cardiac muscle and exists in storage vesicles, which are recruited to the cell surface in response to insulin (Kozka *et al.*, 1995). Although GLUT 1 is found on the cell surface, once GLUT 4 is translocated to the surface it is present in higher concentrations than GLUT 1 and therefore accounts for the transport of the majority of glucose into the cell (Mueckler 1994).

SSAO was found co-localised with GLUT 4 containing vesicles in adipose cells and the increase in glucose transport in the presence of tyramine suggested that SSAO may be

involved in the recruitment of the glucose transporter to the cell membrane (Enrique-Tarancón *et al.*, 1998). SSAO was shown to increase the recruitment of GLUT 4 to the cell surface in the presence of the substrate benzylamine and low concentrations of vanadate. This function was abolished in the presence of semicarbazide and/or catalase suggesting that the H_2O_2 product of the oxidation of benzylamine is the contributing factor (Enrique-Tarancón *et al.*, 1998). Benzylamine and tyramine in association with vanadate stimulated the phosphorylation of a tyrosine residue on two insulin receptors (IRS), whereas benzylamine and vanadate alone had only a weak phosphorylation activity on the IRS. This may be the mechanism by which the GLUT 4 recruitment takes place (Enrique-Tarancón *et al.*, 2000). The combined treatment with benzylamine and vanadate was also shown have anti-diabetic effects in rats (Marti *et al.*, 2001).

In smooth muscle cells, where there are no GLUT 4 glucose transporters, methylamine was shown to increase GLUT 1 translocation to the membrane, which was blocked by catalase, again suggesting a role for H_2O_2 (El Hadri *et al.*, 2002). However vanadate was not necessary to stimulate glucose uptake in this system.

- **Elastin Maturation**

Treatment with SSAO inhibitors resulted in the disruption of elastin architecture in the aorta of rats, along with a lowering of mature elastin and increased dilation of thoracic aortic rings (Langford *et al.*, 1999). Further studies by the same group correlated the production of the extracellular matrix (ECM) to a peak of SSAO activity in cultured smooth muscle cells (SMC). Furthermore, the addition of SSAO inhibitors and alternative substrates (benzylamine) resulted in changes in the composition of the matrix components, collagen and elastin deposits, suggesting a role for SSAO in the protection or modulation of the ECM during a pathological insult (Langford *et al.*, 2002).

- **Role in tooth development**

A novel form of SSAO, which catalyses the oxidative deamination of 5-hydroxytrypamine (5-HT), was discovered in dental pulp, which in all other mammalian tissues is oxidised by MAO. To date, this is the only SSAO found to oxidise 5-HT (Norqvist *et al.*, 1982). Modulation of 5-HT by SSAO suggests a possible role in the inflammatory response in this tissue. SSAO has been localised within teeth to the odontoblast layer, nerve fibres and blood vessels, the presence of the enzyme within nerve fibres may indicate a function as a neurotransmitter metabolising enzyme within dental pulp (O'Sullivan *et al.*, 2002).

- **Post-translational modification of proteins**

A 50 kDa poly-lysine, lysozyme and ribonuclease have been post-translationally modified by SSAO. The modification is believed to be the oxidation of free amino groups on the proteins (Wang *et al.*, 1996).

- **Amiloride- binding protein**

SSAO has been reported to bind the drug amiloride and some other imidazolines (Novotny *et al.*, 1994), although the significance of this is unclear. However, SSAO may have a function as a drug-binding enzyme.

- **Modification of Neuronal K^+ channel function**

Modulation of neuronal K^+ channel currents in a cell line by SSAO was observed. SSAO appears to enhance the channel currents by preventing ceruloplasmin (E.C. 1.16.3.1) induced membrane depolarisation in a time dependent manner. SSAO is therefore believed to be an alternative modulator of neuronal K^+ channels similar to ceruloplasmin but utilising a different mechanism (Wu *et al.*, 1996).

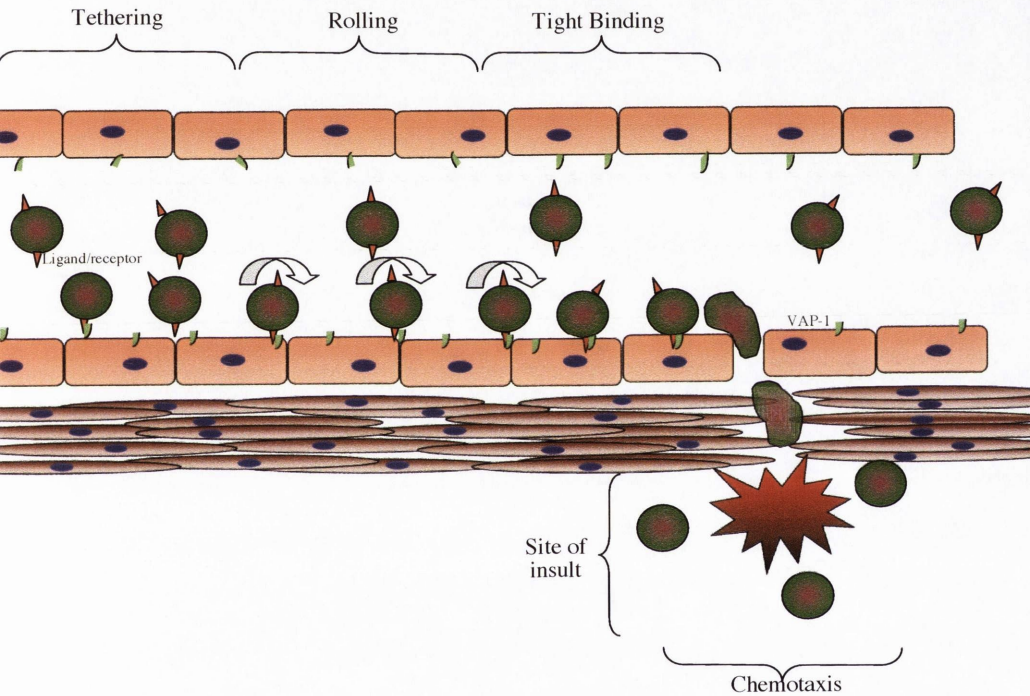
➢ Vascular adhesion

In 1992 a membrane-bound protein believed to be involved in cell adhesion and migration was discovered and designated vascular adhesion protein 1 (VAP-1) (Salmi & Jalkanen 1992). This novel protein functions during an inflammatory response allowing lymphocytes to bind to the endothelial cell wall and facilitates their migration to a site of injury, see Fig. 1.15 (Salmi & Jalkanen 1992). In an attempt to clone this protein, researchers from the same group discovered that VAP-1 had an extremely high homology with members of the copper amine oxidase family (see Section 1.3.9). Characterisation of VAP-1 revealed a quinone co-factor, benzylamine oxidase activity, which was susceptible to inhibition by semicarbazide and hydroxylamine (Bono *et al.*, 1998). Further analysis of the catalytic domain of VAP-1 revealed the presence of the TPQ co-factor confirming the correct designation was as an amine oxidase (Salminen *et al* 1998). The VAP-1 molecule is a transmembrane protein of approximately 84.6 kDa, with a single transmembrane domain at the NH_2 terminal. The protein exists *in-vivo* predominately as a homodimer of 180 kDa (Smith *et al.*, 1998). VAP-1 was found expressed in smooth muscle tissue in addition to endothelial cells and was found to exist in a trimeric form, as well as the dimeric form. Only the dimeric form is found expressed in endothelial cells (Jaakkola *et al.*, 1999). Determination of the specificity for VAP-1 mediated adhesion showed that VAP-1 allows the initial binding of CD8-positive and natural killer cells (NKCs) to peripheral lymph node (PLN) high endothelial venules (HEVs) but this binding also required a peripheral lymph node addressin (PNAd) step (Salmi *et al.*, 1998). VAP-1 demonstrated selectivity by discriminating between CD8- and CD16- positive effector cells (Salmi *et al.*, 1997). Transfection of VAP-1 cDNA into an endothelial cell line, which does not support cell adhesion, rendered the cell line capable of binding lymphocytes under shear and adhesion function was up regulated by CD44 (Salmi *et al.*, 2000). Recent data shows that VAP-1 functions as a molecular brake *in vivo* during granulocyte rolling allowing recruitment (Tohka *et al.*, 2001).

An increased level of VAP-1 has been found in patients with liver disease (Kurkijärvi *et al.*, 1998) and is induced in diabetic insulitis in the mouse (Bono *et al.*, 1999). VAP-1 has

been implicated in mediating leukocyte binding to ischemic heart in humans (Jaakkola *et al.*, 2000) and leukocyte binding in transplanted kidney thereby promoting organ rejection (Kurkijärvi *et al.*, 2001), suggesting that blocking of VAP-1 mediated adhesion may prevent such destruction and rejection respectively.

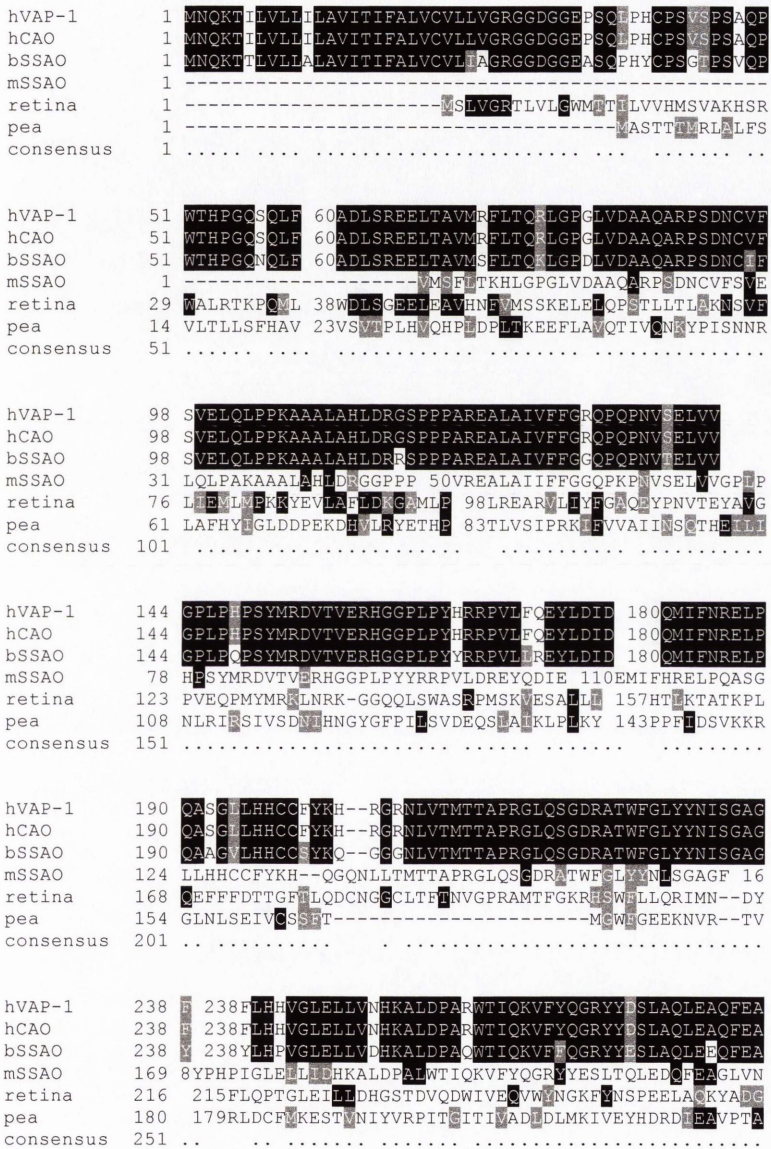
Fig. 1.15. Postulated role of SSAO/VAP-1 in the extravasation of leukocytes to a site of injury or insult. The adhesion cascade is a multistep process involving the various steps depicted. SSAO/VAP-1 mediates the leukocyte rolling and possible contributes to the extravasation of the cells into the tissue.

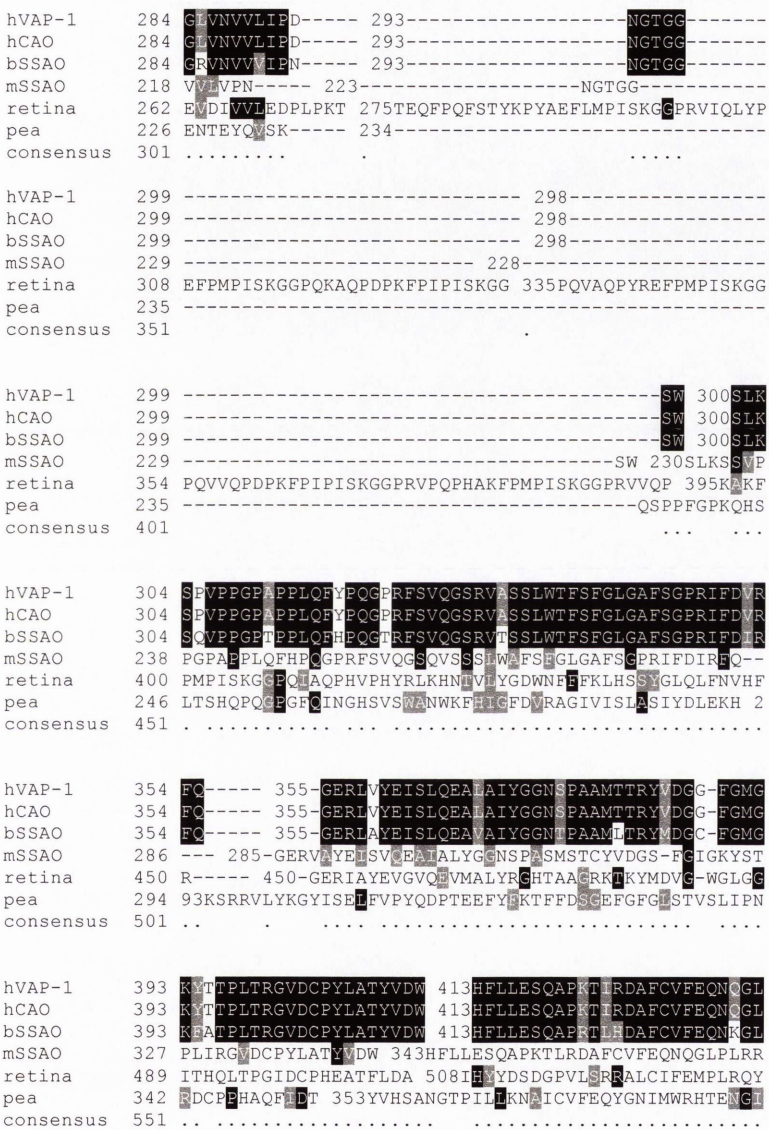

Tethering
Rolling
Tight Binding
Ligand/receptor
VAP-1
Site of insult
Chemotaxis

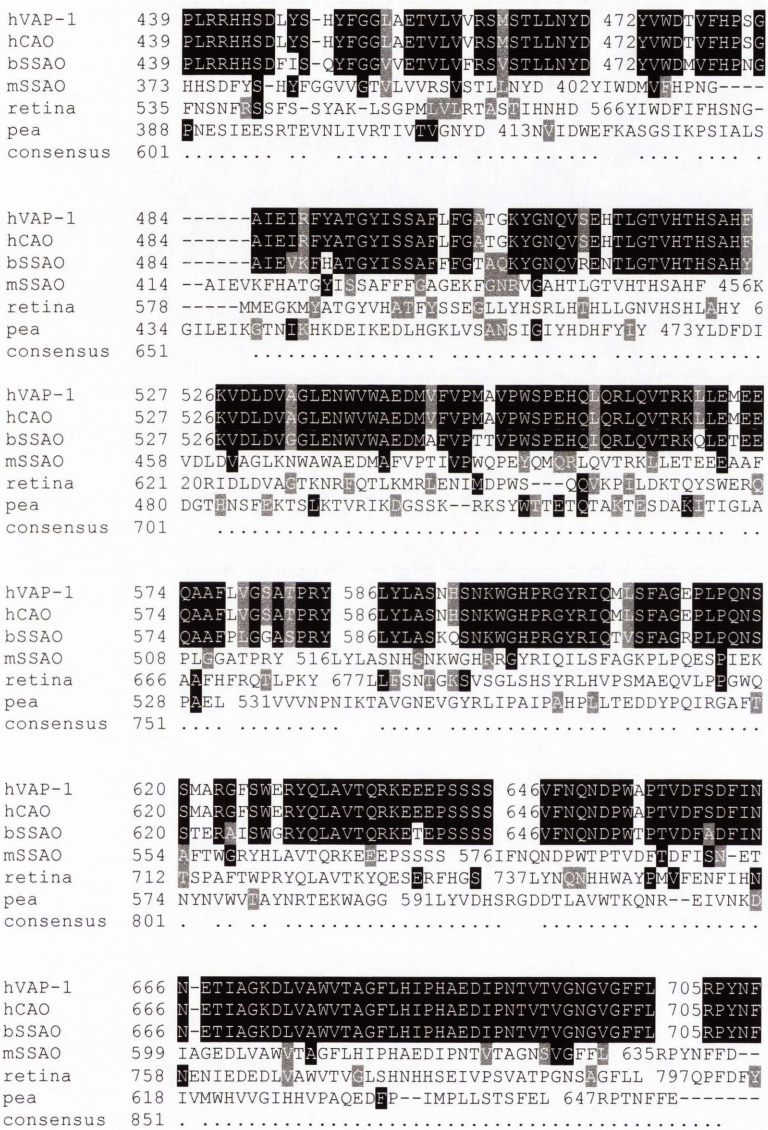
1.3.9 Sequence alignment of SSAO

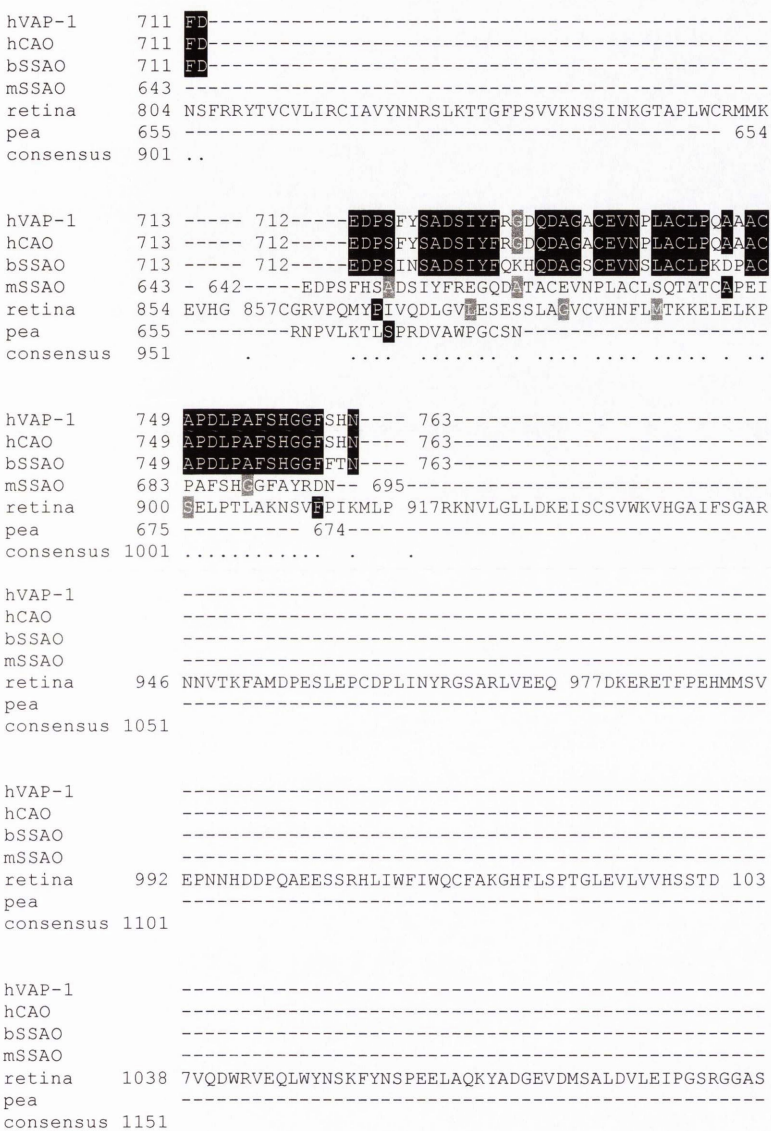
Significant homology has been shown to exist in several isolated and cloned SSAOs. In Fig. 1.16 a representative selection of SSAOs is shown to illustrate the sequence homology that can exist between enzymes from different sources. Two sequences from retina and pea seedling are shown as examples of SSAOs, which are cooper and TOPA containing but show little sequence homology to other SSAOs. DNA sequences were obtained from the National Centre for Biotechnological Information (NCBI) browser ENTREZ PubMed (http://www.ncbi.nlm.nih.gov/PubMed/). DNA sequences were then translated into amino acid sequences using the translation tool on the ExPASy server (http://ca.expasy.org/tools/#translate). Comparison of the sequence homology was determined by submitting sequences in a FASTA format to a multiple sequence alignment program ClustalW (http://www.ebi.ac.uk/clustalw/) and finally to enable shading the alignment was converted using BOXSHADE 3.21 at (http://www.ch.embnet.org/software/BOX_form.html)

Fig. 1.16 Comparison and multiple alignment the semicarbazide sensitive amine oxidases; hVAP-1 (human vascular adhesion protein); hCAO (human copper amine oxidase); bSSAO (bovine semicarbazide-sensitive amine oxidase); mSSAO (mouse semicarbazide-sensitive amine oxidase); human retina and pea seedling amine oxidase.


hVAP-1 1 MNQKTILVLLILAVITIFALVCVLLVGRGGDGGEPSQLPHCPSVSPSAQP
hCAO 1 MNQKTILVLLILAVITIFALVCVLLVGRGGDGGEPSQLPHCPSVSPSAQP
bSSAO 1 MNQKTTLVLLALAVITIFALVCVLLAGRGGDGGEASQPHYCPSGTPSVQP
mSSAO 1 ----------
retina 1 ----------MSLVGRTLVLGWMATILVVHMSVAKHSR
pea 1 ----------MASTTTMRLALFS
consensus 1 ....
hVAP-1 51 WTHPGQSQLF 60ADLSREELTAVMRFLTQRLGPGLVDAAQARPSDNCVF
hCAO 51 WTHPGQSQLF 60ADLSREELTAVMRFLTQRLGPGLVDAAQARPSDNCVF
bSSAO 51 WTHPGQNQLF 60ADLSREELTAVMSFLTQKLGPDLVDAAQARPSDNCIF
mSSAO 1 ----------VMSFLTKHLGPGLVDAAQARPSDNCVFSVE
retina 29 WALRTKPQML 38WDLSGEELEAVHNFVMSSKELELQPSTLLTLAKNSVF
pea 14 VLTLLSFHAV 23VSVTPLHVQHPLDPLTKEEFLAVQTIVQNKYPISNNR
consensus 51 ....
hVAP-1 98 SVELQLPPKAAALAHLDRGSPPPAREALAIVFFGRQPQPNVSELVV
hCAO 98 SVELQLPPKAAALAHLDRGSPPPAREALAIVFFGRQPQPNVSELVV
bSSAO 98 SVELQLPPKAAALAHLDRRSPPPAREALAIVFFGGQPQPNVTELVV
mSSAO 31 LQLPAKAAALAHLDRGGPPP 50VREALAIIFFGGQPKPNVSELVVGPLP
retina 76 LIEMLMEKKYEVLAFLDKGAMLP 98LREARVLIYFGAQEYPNVTEYAVG
pea 61 LAFHYIGLDDPEKDHVLRYETHP 83TLVSIPRKIEVVAIINSQTHEILI
consensus 101 ....
hVAP-1 144 GPLPHPSYMRDVTVERHGGPLPYHRRPVLFQEYLDID 180QMIFNRELP
hCAO 144 GPLPHPSYMRDVTVERHGGPLPYHRRPVLFQEYLDID 180QMIFNRELP
bSSAO 144 GPLPQPSYMRDVTVERHGGPLPYYRRPVLIREYLDID 180QMIFNRELP
mSSAO 78 HPSYMRDVTVERHGGPLPYYRRPVLDREYQDIE 110EMIFHRELPQASG
retina 123 PVEQPMYMRKLNRK-GGQQLSWASRPMSKWESALLL 157HTLKTATKPL
pea 108 NLRIRSIVSDYIHNGYGFPILSVDEQSLAIKLPLKY 143PPFIDSVKKR
consensus 151 ....
hVAP-1 190 QASGLLHHCCFYKH--RGRNLVTMTTAPRGLQSGDRATWFGLYYNISGAG
hCAO 190 QASGLLHHCCFYKH--RGRNLVTMTTAPRGLQSGDRATWFGLYYNISGAG
bSSAO 190 QAAGVLHHCCSYKQ--GGGNLVTMTTAPRGLQSGDRATWFGLYYNISGAG
mSSAO 124 LLHHCCFYKH--QGQNLLTMTTAPRGLQSGDRATWFGLYYNLSGAGF 16
retina 168 QEFFFDTTGFTLQDCNGGCLTFTNVGPRAMTFGKRHSWFLLQRIMN--DY
pea 154 GLNLSEIVCSSFT----------MGWFGEEKNVR--TV
consensus 201 .. ..
hVAP-1 238 F 238FLHHVGLELLVNHKALDPARWTIQKVFYQGRYYDSLAQLEAQFEA
hCAO 238 F 238FLHHVGLELLVNHKALDPARWTIQKVFYQGRYYDSLAQLEAQFEA
bSSAO 238 Y 238YLHPVGLELLVDHKALDPAQWTIQKVFTQGRYYESLAQLEEQFEA
mSSAO 169 8YPHPIGLELLIDHKALDPALWTIQKVFYQGRYYESLTQLEDQFEAGLVN
retina 216 215FLQPTGLEILLDHGSTDVQDWIVEQVWYNGKFYNSPEELAQKYADG
pea 180 179RLDCFYKESTVNIYVRPITGITIVADLDLMKIVEYHDRDIEAVPTA
consensus 251 .. ..


hVAP-1 284 GLVNVVLIPD----- 293----------NGTGG-------
hCAO 284 GLVNVVLIPD----- 293----------NGTGG-------
bSSAO 284 GRVNVVVIPN----- 293----------NGTGG-------
mSSAO 218 VVLVPN----- 223----------NGTGG----------
retina 262 EVDIVVLEDPLPKT 275TEQFPQFSTYKPYAEFLMPISKGGPRVIQLYP
pea 226 ENTEYQVSK----- 234----------
consensus 301 ....
hVAP-1 299 ---------- 298----------
hCAO 299 ---------- 298----------
bSSAO 299 ---------- 298----------
mSSAO 229 ---------- 228----------
retina 308 EFPMPISKGGPQKAQPDPKFPIPISKGGG 335PQVAQPYREFPMPISKGGG
pea 235 ----------
consensus 351 .
hVAP-1 299 ----------SW 300SLK
hCAO 299 ----------SW 300SLK
bSSAO 299 ----------SW 300SLK
mSSAO 229 ----------SW 230SLKSSVP
retina 354 PQVVQPDPKFPIPISKGGPRVPQPHAKFPMPISKGGPRVVQP 395KAKF
pea 235 ----------QSPPFGPKQHS
consensus 401 ... ...
hVAP-1 304 SPVPPGPAPPLQFYPQGPRFSVQGSRVASSLWTFSFGLGAFSGPRIFDVR
hCAO 304 SPVPPGPAPPLQFYPQGPRFSVQGSRVASSLWTFSFGLGAFSGPRIFDVR
bSSAO 304 SQVPPGPTPPLQFHPQGTRFSVQGSRVTSSLWTFSFGLGAFSGPRIFDIR
mSSAO 238 PGPAPPLQFHPQGPRFSVQGSQVSSSLWAFSFGLGAFSGPRIFDIRFQ--
retina 400 PMPISKGGPQAQPHVPHYRLKHNTVLYGDWNFTFKLHSSYGLQLFNVHF
pea 246 LTSHQPQGPGFQINGHSVSWANWKFHIGFDVRAGIVISLASIYDLEKH 2
consensus 451 . ...
hVAP-1 354 FQ----- 355-GERIVYEISLQEALAIYGGNSPAAMTTRYVDGG-FGMG
hCAO 354 FQ----- 355-GERIVYEISLQEALAIYGGNSPAAMTTRYVDGG-FGMG
bSSAO 354 FQ----- 355-GERIAYEISLQEAVAIYGGNTPAAMLTRYMDGC-FGMG
mSSAO 286 --- 285-GERVAYEISVQEAIALYGGNSPASMSTCYVDGS-FGIGKYST
retina 450 R----- 450-GERIAYEVGVQEVMALYRGHTAAGRKTKYMDVG-WGLGG
pea 294 93KSRRVLYKGYISELFVPYQDPTEEFYFKTFFDSGEFGFGLSTVSLIPN
consensus 501 .. . .
hVAP-1 393 KYTTPLTRGVDCPYLATYVDW 413HFLLESQAPKTIRDAFCVFEQNQGL
hCAO 393 KYTTPLTRGVDCPYLATYVDW 413HFLLESQAPKTIRDAFCVFEQNQGL
bSSAO 393 KFATPLTRGVDCPYLATYVDW 413HFLLESQAPRTLHDAFCVFEQNKGL
mSSAO 327 PLIRGVDCPYLATYVDW 343HFLLESQAPKTLRDAFCVFEQNQGLPLRR
retina 489 ITHQLTPGIDCPHEATFLDA 508IHYDSDGPVLSRRALCIFEMPLRQY
pea 342 RDCPEHAQFILT 353YVHSANGTPILLKNAICVFEQYGNIMWRHTENGI
consensus 551 .. ..




hVAP-1 711 FD
hCAO 711 FD
bSSAO 711 FD
mSSAO 643
retina 804 NSFRRYTVCVLIRCIAVYNNRSLKTTGFPSVVKNSSINKGTAPLWCRMMK
pea 655 654
consensus 901 ..
hVAP-1 713 ----- 712-----EDPSFYSADSIYFRGDQDAGACEVNPLACLPQAAAC
hCAO 713 ----- 712-----EDPSFYSADSIYFRGDQDAGACEVNPLACLPQAAAC
bSSAO 713 ----- 712-----EDPSINSADSIYFQKHQDAGSCEVNSLACLPKDPAC
mSSAO 643 - 642-----EDPSFHSADSIYFREGQDATACEVNPLACLSQTATCAPEI
retina 854 EVHG 857CGRVPQMYPIVQDLGVPESESSLACGVCVHNFLVTKKELELKP
pea 655 ----------RNPVLKTLSPRDVAWPGCSN----------
consensus 951
hVAP-1 749 APDLPAFSHGGFSHN---- 763----------
hCAO 749 APDLPAFSHGGFSHN---- 763----------
bSSAO 749 APDLPAFSHGGFFTN---- 763----------
mSSAO 683 PAFSHCGFAYRDN-- 695----------
retina 900 SELPTLAKNSVFPIKMLP 917RKNVLGLLDKEISCSVWKVHGAIFSGAR
pea 675 ---------- 674----------
consensus 1001
hVAP-1 ----------
hCAO ----------
bSSAO ----------
mSSAO ----------
retina 946 NNVTKFAMDPESLEPCDPLINYRGSARLVEEQ 977DKERETFPEHMMSV
pea ----------
consensus 1051
hVAP-1 ----------
hCAO ----------
bSSAO ----------
mSSAO ----------
retina 992 EPNNHDDPQAEESSRHLIWFIWQCFAKGHFLSPTGLEVLVVHSSTD 103
pea ----------
consensus 1101
hVAP-1 ----------
hCAO ----------
bSSAO ----------
mSSAO ----------
retina 1038 7VQDWRVEQLWYNSKFYNSPEELAQKYADGEVDMSALDVLEIPGSRGGAS
pea ----------
consensus 1151

```
hVAP-1          --------------------------------
hCAO            --------------------------------
bSSAO           --------------------------------
mSSAO           ----------------------------
retina     1087 VPVLAPSTKNS 1097FQTLKLEDITRLWCLRHHLVPPTQHSLK 1125
pea             ----------------------
consensus  1201                                 ..............*....
```

1.5 Aims of thesis

Since the discovery that some glycolytic enzymes were also forming lens crystallin as a "part time job", separate from their main perceived function (Farnsworth *et al.*, 1988, Wistow *et al.*, 1988), it should come as no surprise when other multifunctional enzymes are reported. Many such enzymes are continually coming to light, examples include glucose-6-phosphate isomerase is also a major self antigen in rheumatoid arthritis (Matsumoto *et al.*, 1999), acetylcholinesterase is also involved in receptor modulation (Day & Greenfield 2002) and dipeptidyl peptidase IV has been identified as a CD26 adhesion protein (Aytac *et al.*, 2001).

A multifunctional protein with an extensive array of talents, such as SSAO allows for the examination of an interdependency of these functions and this was an aim of this work. With the attention focused on the varying functions of SSAO it would also be prudent to examine any multifunctional abilities associated with MAO, as both enzymes produce similar products, which are implicated in many of SSAO functions. Amine oxidases also have an important pharmacological role in disease states, as previously described, allowing for an analysis of any interaction between pharmacologically important compounds.

This study will focus on the following areas:

- Described as a vascular adhesion protein, VAP-1 was found to exhibit SSAO activity and further work showed it is indeed a member of the SSAO family. What is of interest in this situation is the link between the enzymatic activity of VAP-1 its role on adhesion. Previous work shows that SSAO inhibitors reduce the adhesion of leukocytes, suggesting a link. This study will focus on possible cellular targets for SSAO mediated adhesion, amino sugars, which are abundant in cell-cell interactions and attempt to elucidate their role in adhesion function.

- SSAO has a now recognised involvement in the modulation of glucose uptake, most notably in adipocytes, through the H_2O_2 produced during the

oxidative deamination of amines. What has yet to be clarified is the physiological targets for the enzyme as many studies have used the exogenous substrate benzylamine, albeit to good effect. Another aspect of this study will be to examine the possible involvement of MAO in this situation as both isoforms of the enzyme exist within adipocytes.

- Previously mentioned throughout was the role of inhibitors of both SSAO and MAO and how they can effect the physiological function of both enzymes and, in turn, many downstream events. Inhibitors of these enzymes are now in common use as drugs in a variety of conditions in an attempt to modulate the effects of several disease states. Attempts to try and further elucidate novel amine oxidase inhibitors will be made, based on structural similarities and literature reports, and effects on the activities of the enzymes will be examined along with any physiological role.

Chapter 2
General Methods

2.1 Main Suppliers

Amersham

Amersham PLC
through
P.J. Brennan & Co. Ltd.,
Stillorgan Industrial Estate,
Stillorgan, Co. Dublin
Ireland

BDH

BDH Chemicals Ltd.,
Poole
Dorset
England

Lennox

Lennox Laboratories Ltd.,
J.F.K. Drive,
Dublin 12

MWG

MWG Biotech
Anzinger Strasse 7A
D-85560 Ebersberg, Germany

Sigma

Sigma-Aldrich Ireland Ltd.,
Airton Road,
Tallaght,
Dublin 24,
Ireland

New England Biolabs

New England Biolabs (UK) Ltd.,
73 Knowl Piece, Wilbury Way,
Hitchin, Hertfordshire,
SG4 OTY
England, UK

Promega

Medical Supply Co. Ltd.
Damastown
Mulhuddart
Dublin 15
Ireland

Cruinn Technologies

Cruinn Technologies
Unit 6 Phase 2
Western Industrial estate
Knockmitten Lane
Dublin 12
Ireland

2.1.1 Source of reagents

Product	Supplier
Radiochemicals	
[7-^{14}C] Benzylamine hydrochloride (Specific activity 59 mCi/mmol)	Amersham P.L.C
5-Hydoxy [side-chain 2-^{14}C]tryptamine creatine sulphate (Specific activity 57 mCi/mmol)	Amersham P.L.C
2-Phenylethylamine [ethyl-1-^{14}C] hydrochloride (Specific activity 57 mCi/mmol)	Amersham P.L.C
Tyramine hydrochloride [1-^{14}C] (Specific activity 50 mCi/mmol)	NEN Boston
2-deoxy-D-glucose [1,2-^{3}H] (Specific activity 60 mCi/mmol)	MP Biomedicals
Inhibitors	
Semicarbazide hydrochloride	Sigma Chemicals
Clorgyline hydrochloride	Sigma Chemicals
Deprenyl chloride	Fluka
Substrates	
Benzylamine hydrochloride	Sigma Chemicals
5-Hydroxytrypamine creatine sulphate	Sigma Chemicals
2-Phenylethylamine hydrochloride	Sigma Chemicals
2-deoxy-D-glucose	Sigma Chemicals
(-)-Adrenaline hydrochloride	Sigma Chemicals
(±)-Adrenaline hydrochloride	Sigma Chemicals
Noradrenaline hydrochloride	Sigma Chemicals

Miscellaneous Reagents

2,5-Diphenyloxazole (PPO)	Sigma Chemicals Chemicals
Filter Count	Packard Biosciences
Toluene	BDH Chemicals
Bovine serum albumin (BSA fraction V)	Sigma Chemicals
Citric acid	BDH Chemicals

All other general laboratory reagents were purchased from BDH Chemicals, Lennox Laboratories or Sigma Chemicals.

2.2 Preparation of solutions

All compounds were weighed using a Mettler Model K7T top-loading balance (1 g – 800 g) or a Mettler College 4 place balance (0.0001 g – 1 g).

1 µl to 5 ml volumes were dispensed using a range of Gilson Pipetmann automatic pipettes. All pipettes were checked and calibrated at regular intervals as per the manufacturers instructions.

All solutions were prepared with distilled water (unless stated), which had been de-ionised by passage through a Millipore Milli-U10 water purification system.

The pH of prepared solutions was checked and adjusted if required to the desired pH using a Corning 240 pH meter. The pH meter was calibrated at the start of each day using BDH standard buffer solutions of pH 4.0, 7.0 and 10.01

2.3 Protein Determination

2.3.1 Biuret Assay

To determine the protein concentration in tissues where a relatively high concentration was expected the Biuret method (Gornall *et al.*, 1949) was used. Biuret reagent contains 1.5 g of copper sulphate and 6 g of sodium potassium tartrate dissolved in 500 ml of deionised water. To this solution 300 ml of 10% (w/v) sodium hydroxide was added under constant stirring. The solution was finally diluted to 1 litre with deionised water. Each assay was completed in triplicate where each triplicate contained the sample, 100µl 1.5% (w/v) sodium deoxycholate and was brought up to 1 ml with deionised water. Upon the addition of 4 ml of biuret reagent, the mixture was vortex mixed for 30 s and the reaction allowed to continue for 30 min at room temperature. The absorbance at 540 nm was determined for each triplicate using a Unicam UV-VIS spectrophotometer. A standard curve was determined using bovine-serum albumin (BSA) concentrations ranging from 0-2 mg/ml. From this standard curve the protein concentration could be determined. A representative standard curve is shown in Fig. 2.1.

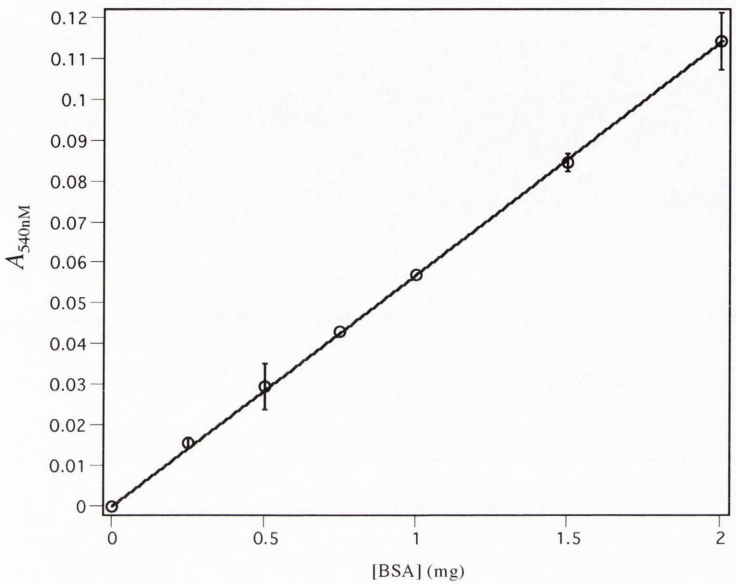


Fig. 2.1. Biuret protein assay standard curve.

Absorbance of known BSA concentrations was determined in triplicate at 540 nm using a Helios UV-VIS spectrophotometer. All data points are mean values ± S.E.M. of at least three replicates.

2.3.2 Markwell method

In the cases where greater sensitivity was required than afforded by the Biuret method, the protein concentration was determined by using the Markwell assay (Markwell *et al.*, 1978). The following mixtures were used:

Reagent A	2% (w/v) sodium carbonate 0.4% (w/v) sodium hydroxide 0.16% (w/v) sodium potassium tartrate 1% (w/v) SDS
Reagent B	6% (w/v) copper sulphate ($CuSO_4.5H_2O$)
Reagent C	100 volumes of Reagent A 1 volume Reagent B
Reagent D	Folin and Ciocalteau's reagent 1:1(w/v) with de-ionised H_2O

Samples of various dilutions were made up to 1ml with de-ionised H_2O. Three ml of Reagent C was added to each sample, mixed using a vortex and then left to stand at room temperature for 15 min. Thereafter 0.3 ml of Reagent D was added and again vortex mixed. After a further 45 min at room temperature the absorbance was determined at 660 nm. Standards were processed with a range of BSA amounts, from 0 – 100 μg. A representative standard curve is shown in Fig. 2.2.

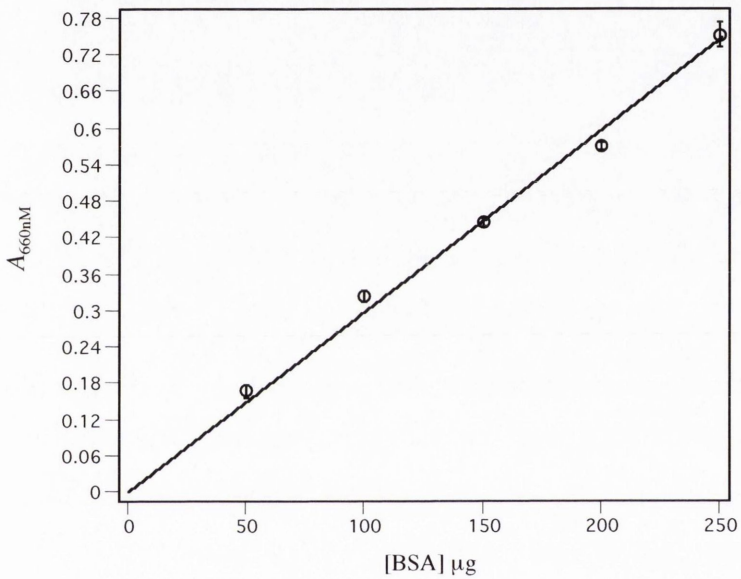


Fig.2.2. Markwell protein assay standard curve.

Absorbance of known BSA concentrations was determined in triplicate at 660 nM using a Helios UV-VIS spectrophotometer. All data points are mean values ± S.E.M. of at least three determinations.

2.3.3 Bio-Rad protein assay

The Bio-Rad protein assay was used to determine protein concentrations and differences within a much smaller range and shorter time than used in the previously described protein assays. The assay is a commercially produced kit (BioRad Laboratories, GmbH, Mucnich, Germany) and is based on dye binding to proteins (Bradford, 1976). The assay was performed as per the manufacturer's instructions. A representative standard curve is shown in Fig. 2.3.

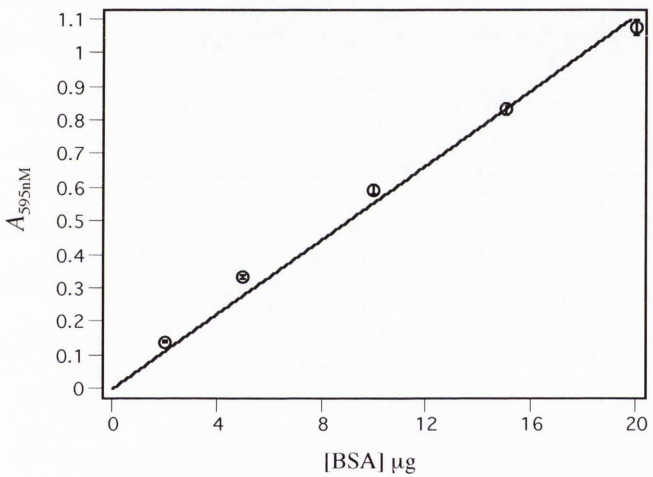


Fig. 2.3. Bio-Rad protein assay standard curve.

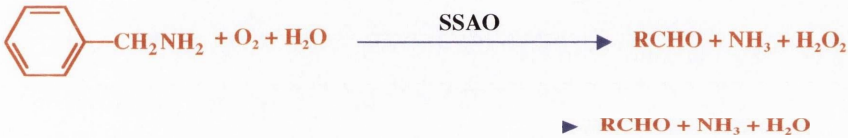
Absorbance of known BSA concentrations was determined in triplicate at 595 nM using a Helios UV-VIS spectrophotometer. All data points are mean values ± S.E.M. of at least three determinations.

2.4 Enzyme activity assays

All enzyme assays were performed at 37 ^{O}C and at pH 7.2, unless otherwise stated.

2.4.1 Spectrophotometric assay of the deamination of benzylamine by SSAO

This assay is based on the conversion of the substrate benzylamine to benzaldehyde, which results in an increase in absorbance at 250nm.



SSAO activity towards benzylamine was assayed directly by a modification of the method of Tabor *et al.* (1954). The standard reaction mixture contained 50mM potassium phosphate buffer, pH 7.2, and 0.024mg/ml SSAO in a final volume of 1ml. The reaction was initiated by the addition of the substrate benzylamine and the rate of change of absorbance at 250nm was monitored using a Cary 300-Bio spectrophotometer. The molar extinction coefficient for benzaldehyde is 13800 $mol^{-1}cm^{-1}$.

2.4.2 Spectrophotometric assay of the deamination of methylamine by SSAO

The oxidation of methylamine can be determined by coupling the formation of formaldehyde to the reduction of NAD^+ in the presence of formaldehyde dehydrogenase (FDH) (Lizcano *et al.*, 2000). The reaction mixture contained 0.1 unit of formaldehyde dehydrogenase (1 unit of FDH will oxidise 1μmol of formaldehyde to formic acid per min at pH 7.5 at 37 ^{O}C), 200 μM NAD^+ and substrate. The reaction volume was brought to 1 ml by adding 50 mM potassium phosphate buffer, pH 7.2 The formation of NADH can be followed on a spectrophotometer at 340 nm. The molar extinction coefficient for NADH is 6220 $mol^{-1}cm^{-1}$.

2.4.3 Hydrogen Peroxide Detection

The oxidation of amines by both SSAO and MAO results in the generation of aldehyde, ammonia and hydrogen peroxide (H_2O_2). Therefore the increase in H_2O_2 can be useful in determining the activity towards any amine oxidase substrate. The formation of H_2O_2 can be determined fluorimetrically (Tipton 1969) or by the spectrophotometric procedure of Holt *et al.* (1997). The reaction mixture for the fluorimetric method comprised potassium buffer, pH 7.2 containing 100μM homovanillic acid (HVA) and 6 units of horseradish peroxidase (HRP) and an appropriate amount of enzyme. The reaction volume was brought up to 3 ml by the addition of potassium phosphate buffer and was initiated by the addition of the substrate. The fluorescence was monitored at an emission wavelength of 425 nm, and an at an excitation wavelength of 315 nm, using a PerkinElmer luminescence spectrometer. After the fluorescence had stabilised the reaction was started by the addition of substrate and the rate of fluorescence increase was measured .

For the spectrophotometric method a chromogenic solution containing vanillic acid (1 mM), 4-aminoantipyrine (500μM) and peroxidase (4 U ml^{-1}) in 0.2 M potassium phosphate buffer pH 7.6 was prepared freshly. Assays were carried out in 96-well plates containing 50μl of chromogenic solution, 50μl of enzyme preparation and 200μl of amine substrate. Reactions were followed using a SpectraMax 340PC microplate reader at 498 nm. A schematic representation of the assay is shown in Fig. 2.4.

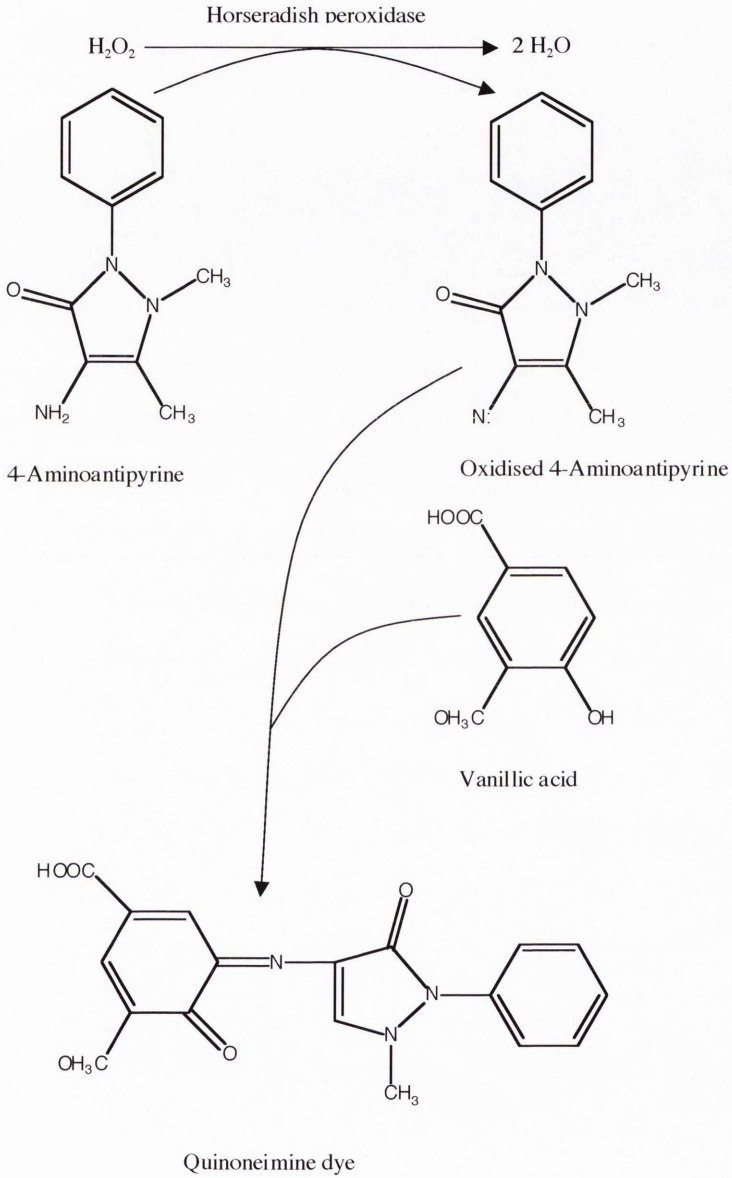


Fig. 2.4 Schematic representation of determination of H_2O_2 assay by the method of Holt *et al.*, 1997

2.4.4 Calculation of radiochemical concentrations

2.4.4.1 Determination of specific activities from d.p.m.

All radiochemical assays were performed using a PerkinElmer TriCarb 2100 scintillation spectrometer which automatically calculated the conversion from c.p.m. to d.p.m. with automatic correction for counting efficiency.

The specific activity (S.A.), i.e. the rate of production formation by an enzyme could be calculated using the d.p.m. measurement obtained from radiochemical assays. Taking into account the extraction efficiency of each substrate (see table 2.1), the duration of the reaction and the final protein concentration the S.A., in pmol.min^{-1}.mg protein^{-1}, can be calculated using the following equation:

S.A. = d.p.m. (100/X)(Y)(1/T)(1/µl of protein per reaction)(1000/ mg/ml protein)

Where X is the extraction efficiency of the substrate, T is the time of the reaction and Y is the conversion factor from d.p.m. to pmol and is calculated from the following equation:

Y= (mmol/mCi)(mCi/2.22 X 10^9 d.p.m.)(10^9 pmol/nmol)

Substrate	Mean extraction efficiency (%)
Benzylamine	86.8
5-HT	74.4
2-PEA	92.5
Tyramine	92.4

Table 2.1 Extraction efficiency of radiolabelled amine substrates in toluene-ethyl acetate PPO 0.6% (w/v) scintillant (Fowler & Oreland, 1980).

2.4.5 Radiochemical assay for SSAO activity

The oxidation of benzylamine by SSAO was also determined using the radiochemical method of Tipton *et al.* (2000). Each assay was completed in triplicate in 6 ml plastic scintillation vials using either bovine plasma amine oxidase (as supplied by Sigma) or partially purified bovine plasma amine oxidase as a source of the enzyme. Each triplicate contained a required concentration of enzyme, 25µl of the substrate, which consisted of both labelled (^{14}C)-labelled ([7-^{14}C]Benzylamine hydrochloride; Amersham) and unlabelled benzylamine mixed to give a final specific activity of 0.3µCi/µmol. The final volume of the assay was brought up to 225µl with 50mM potassium phosphate buffer (pH 7.2). The reaction was stopped by the addition of 100µl of 2M citric acid. Labelled product was extracted in toluene-ethyl acetate, containing 0.6% PPO (w/v) scintillant. After freezing at –80°C for 40 min, the solvent phase, which contained the radioactive product, was removed to fresh vials and radioactivity was measured by scintillation counting. To ensure the reaction was proceeding in an essentially linear manner a time course was determined, see Fig 2.5.

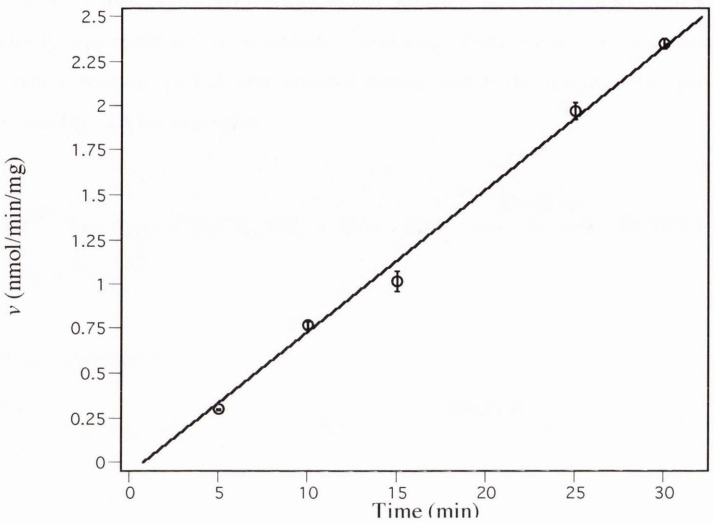
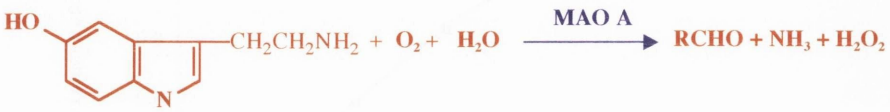


Fig. 2.5. Time course of ^{14}C-benzylamine (20 μM) deamination by SSAO. All data points are mean values ± S.E.M. of at least three replicates.

2.4.6 Radiochemical assay for MAO activity

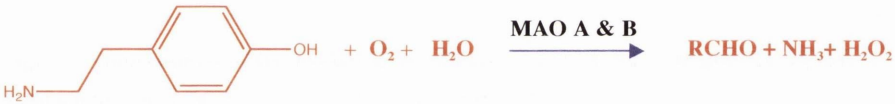
The oxidation of 5-hydroxytrypamine, 2-phenethylamine (substrates for MAO A and MAO B, respectfully) and tyramine (substrate for both MAO isoenzymes) was determined according to the method of Tipton *et al.* (2000). The assay was completed in triplicate in 6 ml plastic scintillation vials using rat liver mitochondria as a source of the enzymes. Each triplicate contained 25μl of rat liver homogenate, 25μl of the appropriate substrate, which consisted of labelled (^{14}C) and unlabelled substrate mixed to give a final specific activity of 0.6 μCi/μmol. The final volume of the assay was brought up to 225μl with 175μl of 100mM potassium phosphate buffer. The reaction was stopped by the addition of 100μl of 2 M citric acid. Labelled product was extracted in toluene-ethyl acetate, containing 0.6% PPO (w/v) scintillant. After freezing at –80°C for 40 min, the

solvent phase, which contained the radioactive product, was removed to fresh vials and radioactivity was measured by scintillation counting. Time courses were determined to ensure that a reaction period was selected during which the reaction was proceeding linearly, see Fig. 2.6 for examples.



5-Hydroxytrypamine

2-Phenethylamine



Tyramine

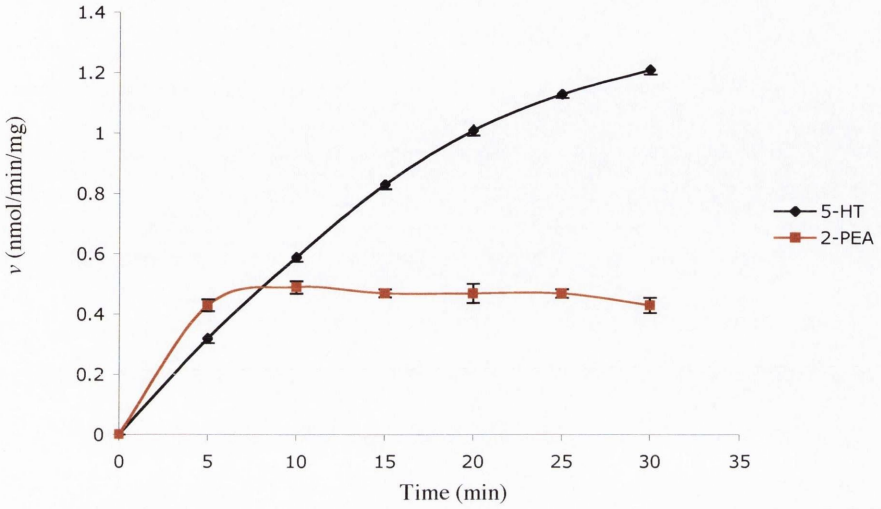


Fig. 2.6. Time courses of the oxidative deamination of ^{14}C 5-HT (100 μM) by MAO A (black line) and ^{14}C 2-PEA (10 μM) by MAO B (red line).

Assay times selected from this data were 10 min for ^{14}C 5-HT, 5 min for ^{14}C 2-PEA. Similar studies showed that the rate of tyramine oxidation was linear for at least 10 min. All data points are mean values ± S.E.M. of at least three replicates.

2.4.7 Determination of the reversibility of SSAO inhibition

Reversibility of inhibition was assessed by dilution experiments. The enzyme (bovine plasma amine oxidase) from Sigma or semi-purified enzyme, at a concentration of 1.2 mg protein.ml^{-1}, was incubated with inhibitor until inhibition was essentially complete, using previous time-course experiments as a guide. 20μl samples were then removed and diluted into the standard benzylamine assay mixture, to give a final protein concentration

of 0.024 $mg.ml^{-1}$, for activity determinations. SSAO activity was then assayed as described above in Section 2.2.1. A control sample was also taken through the same procedure where inhibitor was added to the desired final concentration after dilution.

2.4.8 Determination of the reversibility of MAO inhibition

Reversibility of MAO was assessed by centrifugation and resuspension. 1ml of a 10mg/ml suspension of rat liver mitochondria was incubated for 1 hour with sufficient inhibitor to achieve essentially complete inhibition. The inhibitor was then depleted by centrifugation at 14000 rpm for 10 minutes. The supernatant was discarded and the mitochondrial pellet was resuspended in a fresh phosphate buffer (100 mM, pH 7.5) to give a concentration of approximately 10 mg/ml. The activity of MAO was then assayed as described above in Section 2.3.3. This procedure was completed in triplicate.

2.4.9 Curve fitting and statistical analysis

K_m and V_{max} values were determined by non-linear regression, using the programme MacCurveFit, Version 1.5 (Kevin Raner Software, Australia) to fit the recorded experimental data to the Michaelis-Menten equation:

$$v = \frac{V_{max}[S]}{\left(K_m + [S]\right)}$$

Where v is the observed velocity of the reaction, [S] the substrate concentration, V_{max} the maximum velocity and K_m is the Michaelis constant.

IC_{50} values (the inhibitor concentration that gives 50% inhibition of the reaction) were also determined by using MacCurveFit to fit data to the equation.

$$F(x) = \frac{100\left(10^{(x+a)}\right)}{10^{(x+a)} + 1}$$

2.5 Isolation of bovine plasma amine oxidase (see Fig. 2.1 for flow chart)

The procedure used was a modification of Wang *et al.*, 1994. All operations were performed at 0-4 ^{o}C.

10 L of bovine blood was obtained from the abattoir, mixed with 1L of 2.5% sodium citrate solution and centrifuged at 3000g for 20 min, retaining the plasma.

Ammonium sulphate was added, with continuous stirring, to 4 L of plasma to give 35% saturation. Stirring was continued for 2 h before centrifugation at 10,000*g* for 20 min. The supernatant was retained and ammonium sulphate was added, with continuous stirring, to give 55% saturation. Stirring was continued for 30 min before centrifugation at 10,000*g* for 20 min. The precipitate was retained and dissolved in 200ml of 100mM potassium phosphate buffer, pH 7.2.

A Q-Sepharose column (30 X 2.5 cm) was equilibrated with 10mM potassium phosphate buffer at pH 7.5. The resuspended pellet was applied to the column at a flow rate of 150ml/h. The column was first washed with 500ml of starting 10mM phosphate buffer and then eluted with a linear concentration gradient from 0.05 M to 0.35 M NaCl in the same buffer. The elution rate was 100 ml/h, collecting fractions of 8mL each. The fractions with a specific activity equal or higher than 0.4 μmol/min/mg were retained and pooled.

A Con A-Sepharose column (30 X 2.0 cm) was equilibrated with 100mM potassium phosphate buffer at pH 7.2. The solution containing the pooled fractions from the Q-Sepharose column was diluted 1:1 with starting buffer and applied to the column. 500 ml of 100mM phosphate buffer and then with 300ml of 20mM methyl-α-D-glucopyranoside in 100mM phosphate buffer at pH 7.2 were passed through the column.. The SSAO was then eluted with 200mL of 0.5 M methyl-α-D-mannopyranoside in 100mM potassium phosphate buffer, pH 7.2, whilst collecting fractions of 6 ml. The samples with specific activities equal or higher than 0.95 μmol/min/mg were pooled.

The pooled fractions were then applied to a Q-Sepharose (30 X 2.5 cm) column, previously equilibrated with 20mM potassium phosphate buffer (pH 6.8). The column was then washed with 300ml of this buffer and eluted with a continuous gradient from 0.05 M to 0.35 M NaCl in 20 mM potassium phosphate buffer, pH 6.8. The fractions with

SSAO specific activity higher than or equal to 15 μmol/min/mg were then pooled and dialysed against 20 mM potassium phosphate buffer at pH 6.8 for 6 h, concentrated using a Millipore Centricon, centriplus YM-100 and stored in aliquots of 300μl in 20% glycerol at $-20^{\circ}C$.

Fig. 2.8 shows the elution profile of bovine plasma SSAO and Fig. 2.9 shows a representative SDS-PAGE and a western blot confirmation of the presence of SSAO. The results from a representative purification are summarised in Table 2.2. Km values against benzylamine and methylamine were determined for the partial purified SSAO and compared favourably with values obtained from the pure enzyme obtained from Sigma Chemicals, Pure enzyme values were, 997 ± 59μM for benzylamine, 305 ± 32μM for methylamine. Partially purified enzyme values were 1088 ± 73μM for benzylamine and 317 ± 64μM for methylamine.

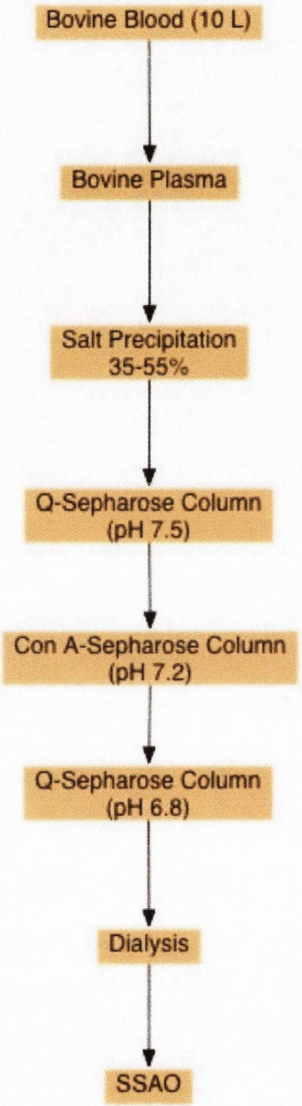
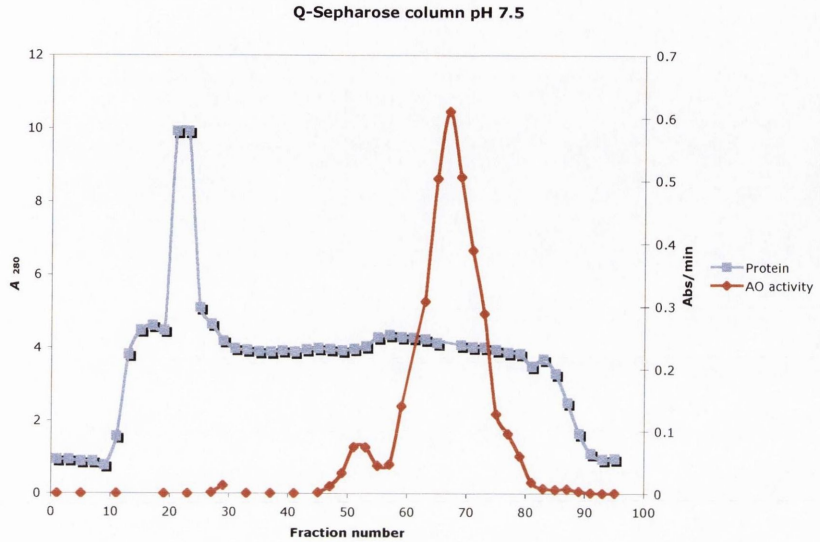
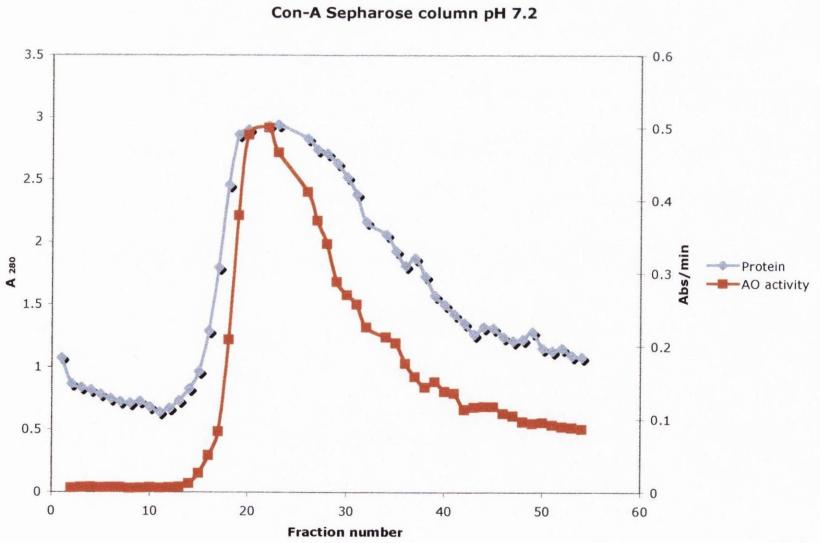


Fig. 2.7. Outline of the bovine-plasma SSAO purification scheme.


Q-Sepharose column pH 7.5
A 280
Abs/min
Protein
AO activity
Fraction number


Con-A Sepharose column pH 7.2
A 280
Abs/min
Protein
AO activity
Fraction number

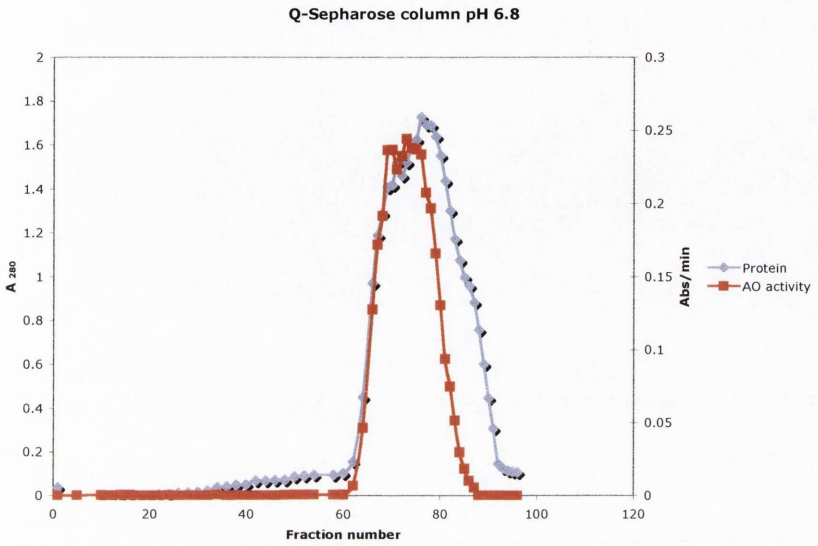


Fig. 2.8. Elution profiles for bovine plasma SSAO activity during purification procedure.

Protein presence was determined by measuring the absorbance at 280nm. SSAO activity was determined as described in Section 2.4.1.

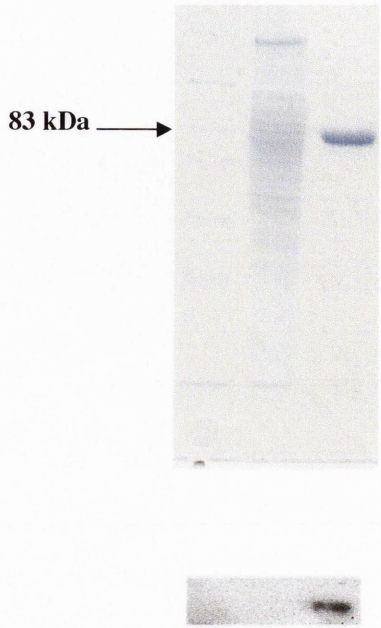


Fig 2.9 SDS-PAGE analysis of SSAO after purification by the method described.

Lane 1, molecular weight markers; lane 2, initial plasma sample (5 μg protein), and lane 3 SSAO after purification. The lower panel shows a western blot completed using method as described in Section 2.12. The primary antibody dilution was a 1/200 dilution of polyclonal antibody raised in rabbit against bovine lung amine oxidase, which was kindly supplied by Dr. Mercedes Unzeta (Autonomous University of Barcelona, Spain).

Purification Step	Volume (ml)	Activity (nmol/min/ml)	Total Activity (nmol/min)	Protein Concentration (mg/ml)	Total Protein (mg)	Specific Activity (nmol/min/mg)	Yield (%)	Purification Fold
Plasma	4500	0.94	4230	5.6	25200	0.29	100	---
Post Con-A	95	2.8	266	3.1	294.5	0.77	6.2	2.6
Post Q-seph pH 6.8	150	13.2	1980	1.7	255	3.2	46	11

Table 2.2. Partial purification of bovine plasma SSAO. 50µl fractions were assayed by the spectrophotometric method describe in section 2.4.1 at a fixed concentration of 5mM benzylamine.

2.6 Preparation of bovine lung microsomes.

A fresh bovine lung was obtained from the abattoir and approximately 300 g was finely chopped into small fragments to which Tris buffer (pH 7.7) containing 10 mM Tris and 250 mM sucrose is added (1 litre / 100g). The lung was then homogenised to homogeneity in a waring blender before filtration through a double layer of cheesecloth. The homogenate was then centrifuged for 10 min at 10,000 g and the supernatant retained and further centrifuged for 10 min at 10,000 g, again the supernatant was retained. $CaCl_2$ was added to the supernatant to final concentration of 10 mM and was mixed for 15 min at 4°C. The supernatant was then centrifuged for 30 min at 25,000 g. The pellet from this step was retained and resuspended in 200 ml of 20 mM potassium phosphate buffer containing 150 mM KCl, pH 7.2. The pellet was then homogenised using a hand held dounce homogeniser. The homogenate was then washed by centrifugation for 30 min at 25,000 g. The pellet was resuspended in 150 ml of 20mM potassium phosphate buffer, pH 7.2. Diluted to a final concentration of 10-15 mg/ml, aliquot and stored at -70°C. Km value for microsomal bound SSAO was 76 ± 29 μM against benzylamine.

2.7 Preparation of Rat Liver Mitochondria

A female Wistar rat (approx. 250 g) was sacrificed by cervical dislocation. The liver was removed and placed in 50 ml of ice-cold Tris (5 mM) - EGTA (1 mM) buffer, pH 7.4, containing sucrose (250 mM). The liver was finely chopped using a scissors and the buffer was changed at least twice to remove any blood. The chopped the liver was then homogenised using a hand-held Thomas homogeniser; applying 5 passes of a 0.026" clearance pestle, followed by five passes of a 0.012" clearance pestle.

The homogenate was centrifuged in a pre-cooled (4°C) Sorvall RC5S centrifuge, using an SS34 rotor, at 3000rpm for 3 minutes. The resulting supernatant was decanted into a new centrifuge tube before centrifugation at 10,000rpm for 10 minutes at 4°C. The supernatant was carefully decanted and discarded and the resulting pellet was homogenised in the sucrose, Tris-EGTA buffer, using five passes with the tighter pestle. This resuspension and centrifugation procedure was repeated a further two times.

After the final centrifugation the pellet was resuspended in 1.0 ml of 100 mM phosphate buffer pH 7.4.A Biuret assay was completed on each mitochondrial preparation to estimate the protein concentration, which was adjusted to a final concentration of 10 mg/ml, aliquots of 1 ml were stored at –20 ^{o}C.

2.8 Preparation of adipocytes from rat white adipose tissue

White adipose tissue was removed from epididymal fat pads of a male Wistar rat of approximately 250 g and stored in Krebs-bicarbonate buffer consisting of the following components, KH_2PO_4 (1.2 mM), $CaCl_2(2H_2O)$ (2.5 mM), $MgSO_4$ (0.6 mM), KCl (4.7 mM), NaCl (118 mM) and $NaHCO_3$ (25 mM), pH 7.5. Any areas of tissue that appeared blood contaminated or contained any vascular components were removed and discarded. The tissue was then finely chopped with a sharp scissors until a smooth paste like consistency was achieved. The tissue was split into test tubes into which a 0.5 mg/ml collagenase in Krebs-bicarbonate buffer, containing 1% BSA, was added. The ratio of tissue to collagenase was approximately 3:10 by volume. After mixing by gentle inversion the tubes were placed into a waterbath at 37^{o}C for 60-90 min, with regular mixing through inversion. Once adipocytes were visible, in the form of oil droplets in the solution, the tubes were removed from the waterbath and the adipocytes were washed at least three times with Krebs-bicarbonate buffer by transferring adipocytes to a fresh test tube before addition of fresh buffer, to remove the collagenase. The adipocytes were then filtered through cheese cloth to remove any remaining particulate matter and finally stored in Krebs-bicarbonate buffer at a ratio of approximately 1:3 by volume, adipocytes: buffer. See Fig. 2.10 for a flow chart of the procedure.

2.9 Glucose uptake determination

These assays were performed, in triplicate, in 2ml Eppendorf tubes. 400µl of Krebs-bicarbonate buffer containing 2% BSA was added to clean tubes. 100µl of freshly prepared adipocytes were suspended on top of the buffer and additions of stimulants or inhibitors were made, as required and incubated at 37^{o}C in a cell- culture incubator for

the appropriate time. After stimulation, ^{3}H-2-deoxy-D-glucose was added and incubation was continued for 15min. Glucose uptake was stopped by filtration of the assay mixture through 8.0μm cellulose nitrate filters. Filters were pre-soaked in Krebs-bicarbonate buffer containing 2% BSA and place on to a Millipore sampling manifold. Cells trapped on the filters were then washed in 10 ml of Krebs-bicarbonate buffer and allowed to air dry for 5 min. Dried filters were placed into 6ml scintillation vials to which 3 ml of Filter-Count (PerkinElmer) was added. The vials were vortex mixed and allowed to stand for 15min to allow the filters to dissolve fully. Radioactivity measurements were then performed in a PerkinElmer TriCarb 2100 scintillation spectrometer.

2.10 Adipocyte plasma membrane preparation

Adipocyte plasma membranes were prepared by a modification of the method of Simpson *et al.* (1983). Adipocytes were prepared from adipose tissue removed from 5 male Wistar rats as described in Section 2.7 and stimulated as described in Section 2.8. Isolated cells were washed twice in TES buffer containing 20mM Tris-HCl, 1mM EDTA and 255mM sucrose and resuspended in 20 ml TES buffer. The cell suspension was then homogenised using a 50ml Teflon homogeniser, with a tight (0.012" clearance) pestle, followed by centrifugation at 16,000*g* for 15 min. The fat cake was carefully removed and the pellet resuspended in TES buffer and further centrifuged at 16,000*g* for 15min. The pellet was resuspended in 5mls of TES buffer. This suspension was then layered on to TES buffer containing 20mM Tris-HCl, 1mM EDTA and 1.12M sucrose and centrifuged at 101,000g for 70 min. The adipocyte plasma membranes were carefully collected at the interface, resuspended in 50mls of TES buffer and centrifuged at 48,000*g* for 45 min. The pellet was then resuspended in Krebs-bicarbonate buffer to give a final protein concentration of 3mg/ml

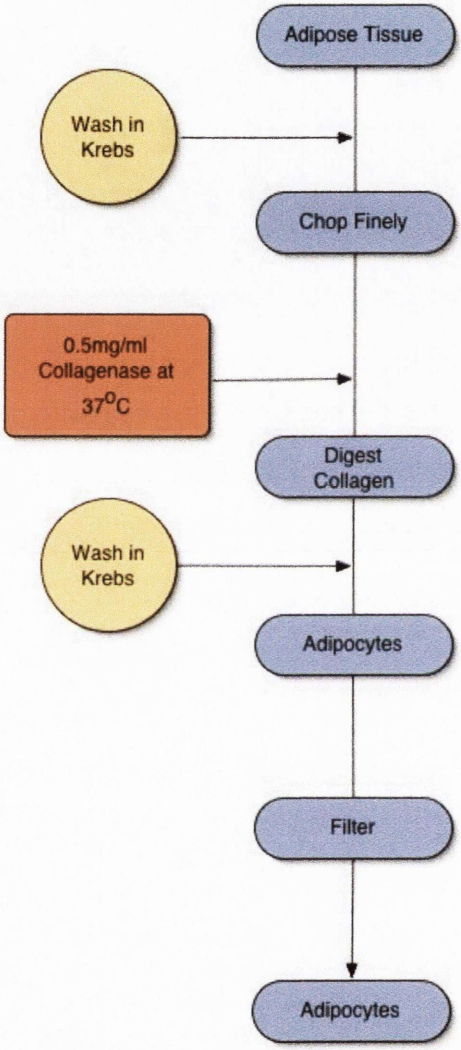


Fig. 2.10. Scheme for adipocyte isolation and preparation. Experimental details are given in the text. Krebs = Krebs-bicarbonate buffer (see Section 2.7)

2.11 Radiochemical assay for amine oxidase activity in adipocytes

The oxidation of tyramine a substrate for SSAO, MAO A and MAO B was determined according to the method of Tipton *et al.* (2000). The assay was completed in triplicate in 6 ml plastic scintillation vials using freshly prepared adipocytes. Each triplicate contained 10µl of adipocytes, 25µl of the substrate, which consisted of labelled (^{14}C) and unlabelled substrate mixed to give a final specific activity of 0.6 µCi/µmol. The final volume of the assay was brought up to 225µl with 175µl of 100 mM potassium phosphate buffer. Where inhibitors were added 30 min prior to the addition of substrate was included in the assay. Other details were as described in Section 2.4.5

2.12 Cytology

Slides of adipocytes were prepared on poly-lysine coated glass slides. 500 µl of Krebs-bicarbonate buffer containing 100µl of freshly-prepared adipocytes were deposited onto the slide by centrifugation on a cytospin at full speed for 3min. The slides, now containing the cells, were air dried for 20min before fixation in 4% formaldehyde for at least 6 hours. Once fixed, the slides were stained using the RapiDiff staining kit (Bios Europe via Langanbach Services Ltd, Co. Wicklow) following the manufacturer's instructions but without the fixation step. After staining, the slides were placed in xylene for 5min and a coverslip containing a drop of DPX mountant was placed directly over the cells and allowed to dry. Adipocytes were then visualised by microscopy.

2.13 Sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE)

2.13.1 Sample preparation

Samples were prepared for electrophoresis by boiling the required amount of protein sample (approximately 5µg) in sample buffer for 5 min. The sample buffer consisted of 2% (w/v) SDS, 0.5% (v/v) 2-mercaptoethanol, 25 mM Tris-HCl, pH 6.8, containing 0.0012% (w/v) bromophenol blue and 10% (v/v) glycerol.

2.13.2 Gel preparation and Electrophoresis

The resolving gel components (see Table 2.2) were mixed and immediately poured into the assembled casting apparatus of a Bio-Rad Mini-PROTEAN II electrophoresis cell (assembled according to the manufacturer's instructions) and overlaid with ethanol to allow polymerisation. When set, the ethanol was removed and the stacking gel was added to which a 1mm Teflon comb with 10 wells was inserted. When set the comb was removed and the wells rinsed out with water to remove any unpolymerised acrylamide. The samples were loaded using a P-20 Gilson pipette. Molecular weight markers (New England Biolabs) appropriate to the sample were loaded into the first and last lanes. The gels were then run at 60 volts for 45 min or until the tracker dye reached the bottom of the gel.

Stock Solutions	Stacking Gel	Resolving Gel (15%)
30% Acrylamide	835µl	3.75ml
2% Bisacrylamide	325µl	325µl
1.0 M Tris-HCl buffer, pH 6.8	650µl	-
1.5 M Tris-HCl buffer, pH 8.7	-	1.875µl
10% SDS	50µl	75µl
Water	2.525ml	1.425µl
10% (w/v) Ammonium persulphate	25µl	25µl
TEMED	20µl	20µl

Table 2.2. Composition of stacking and resolving gels used for 15% SDS-PAGE

2.13.3 Staining and Destaining Gels

When removed from the apparatus the gel was lightly rinsed with distilled water and stained in 0.055% Coomassie blue G-250, 0.027% Coomassie blue R-250, 25% ethanol and 10% acetic acid for 30 min. The gel was then destained in 10% ethanol and 5% acetic acid overnight.

2.14 Western Blot Analysis

SDS PAGE gels were run in duplicate, as described in Section 2.11, using pre-stained broad-range makers from New England Biolabs. The gels were run until the tracker dye reached the bottom of the gel. Upon completion of the run one gel was stained, as described in section 2.11.3. The other gel was equilibrated for 20 min in transfer buffer (see Table 2.3 for composition).

Glycine	192 mM
Tris, pH 8.3	25 mM
SDS	1.3 mM
Methanol	15% (v/v)

Table 2.3. Composition of the transfer buffer used in western blot analysis

The membrane was activated by rinsing in methanol for 10 seconds. Blotting paper and transfer membrane (Millipore Immobilon-P transfer membrane, pore size 0.45 μm) were then placed in transfer buffer for 20 min. Once equilibrated the components were place in the semi-dry blotter (Biorad Transblot semi-dry transfer cell) in the following order, from the bottom: 1 sheet of extra thick blotting paper, transfer membrane, gel and 1 sheet of extra thick blotting paper. Once all the components were in place they were trimmed to the dimensions of the gel and replaced in the blotter. This was run at 7 volts for 15 min. Transfer was confirmed by the appearance of the pre-stained markers on the transfer membrane and by staining of the gel to ensure all proteins were transferred. After transfer the blot was rinsed in Tween 20 (0.1%)-Tris buffered saline (TTBS) and blocked either overnight at 4^{O}C or at room temperature for 90 min in 3% Marvel/TTBS on an orbital shaker. Membranes were incubated after blocking with a suitable dilution of the required primary antibody in 3% Marvel/TTBS for 1 hour. They were then rinsed in TTBS and washed 5 times in TTBS for 15 min each. The membrane was further incubated with the appropriate peroxidase labelled secondary antibody for 1 hour. The blot was rinsed in Super Signal West Pico Chemiluminesent Substrate (Pierce), according to the

manufacturer's instruction, and exposed to Kodak film (X-omat LS film 13 X 18 cm) before development and analysis.

2.15 Induction of C75 black mice with Experimental Autoimmune Encephalomyelitis

For actively induced EAE, female C57 black mice (6-8 weeks old) were immunised with 150 μg of MOG35–55 emulsified in CFA containing 5 mg/ml *Mycobacterium tuberculosis* (Strain H37Ra) subcutaneously and given 500 ng of pertussis toxin on day 0. On day 2 the mice received a further 500 ng of pertussis toxin (Fife *et al.*, 2000). For animals with no clinical signs, onset arbitrarily calculated as one day after the experiment was terminated. Food and water was placed on the floor of the cage for paralysed animals.

Chapter 3

Interaction between SSAO/VAP-1 and amino hexoses: implications for the modulation of cell adhesion.

3 Introduction

3.1 Cell to cell recognition

Cell surfaces contain an abundance of complex carbohydrates which are utilised extensively for cell-cell recognition. The binding can occur when a complex carbohydrate on one cell interacts and recognizes a protein or a carbohydrate with a complementary biding site on another cell. This ability can also allow cells to distinguish neighbouring cells from each other and, thus, exert a regulatory effect on both cellular and systematic functions (Taylor & Drickamer, 2004) The specific family of proteins which act as receptors for such complex carbohydrates are the lectins which are protein or glycoprotein substances capable of specific recognition of, and reversible binding to, carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognised glycosyl ligands. This group also includes monovalent lectins (i.e. bacterial and plant toxins). These lectins bind to sugar moieties on cell walls or membranes and thereby change the physiology of the membrane to cause agglutination, mitosis, or other biochemical changes in the cell.

The carbohydrates in question are usually oligosaccharides, most of which are bonded to proteins, referred to as glycoproteins, while some are covalently bonded to lipids, which are termed glycolipids. Utilizing carbohydrates as the main intermediates in cell recognition conveys the necessary specificity of the reversible contacts, which are essential when the contact between cells needs to be transient, but also allows the flexibility needed when cells are constantly under pressure stress, such as in blood flow (Bucior & Burger 2004). The ability of cells to make specific interactions with another cell allows the tissue to develop and repair throughout its existence. Of course the downside is that invading pathogens can misuse the receptors for their own survival, for example *Bordetella pertussis* may bind glycolipids allowing adherence and therefore infection (Brennan *et al.*, 1991). *Helicobacter pylori* shows high-affinity binding to laminin, the major glycoprotein of the basement membrane (Valkonen *et al.*, 1993). Glycosaminoglycans (GAGs) on cell surfaces and in the extracellular matrix also show binding by several microbial pathogens including *Streptococcus pyogenes* (Frick *et al.*,

2003). Another undesirable effect of this cell-cell recognition is the ability of tumor cells to use the carbohydrate receptors to circulate around the body (Hakomori 2002). Hyaluronan is a cell surface GAG that has been implicated in cancer progression and has recently been shown to facilitate metastasis in colon carcinoma (Laurich *et al.*, 2004). There are normal functions which require cell-cell recognition, such as neurite outgrowth, where $\alpha 3\beta 1$ integrin binds the transmembrane-4-superfamily proteins CD151 and CD81, and selectively contributes to neurite outgrowth (Stipp & Hemler 2000). The most familiar role of carbohydrate mediated cell-cell recognition involves the selectins, which are a family of cell-surface adhesion molecules on leukocytes, and endothelial cells that bind sugar moieties on specific glycoproteins with mucin-like features which contribute to leukocyte rolling (Ley *et al.*, 1995).

3.2 Leukocyte migration

The recruitment of immunological cells, such as leukocytes is a prime example of the specificity and complexity involved in cell-to-cell recognition. In response to injury or infection the immune system can specifically regulate the migration of leukocytes to the site of insult. For this to occur a series of chemical signals are initiated that result in the promotion of a cellular response to enable repair at a site of injury or infection. Leukocyte trafficking involves crossing the endothelium lining the capillaries. Crossing the endothelium is called extravasation and depends on leukocyte-specific cell adhesion molecules (CAMs). CAMs belong to four families of molecules: selectins, mucins, integrins and the Ig supergene family. Selectins contain a distal lectin domain that recognizes specific carbohydrates, usually those that are sialyated. Selectins are found on both leukcoytes and endothelium. Mucins are serine and threonine rich proteins which are heavily glycosylated and are specifically recognised as ligands by selectins (Lasky 1992). Integrins which are found on many different leukocytes are hetrodimeric proteins composed of α and β chains, their ligands are the CAMs of the Ig superfamily. Immunoglobulin superfamily members contain different numbers of Ig homology domains and are found on vascular endothelium and are ligands for integrins. See Fig. 3.1 for a description of the roles of the various molecules involved in leukocyte trafficking.

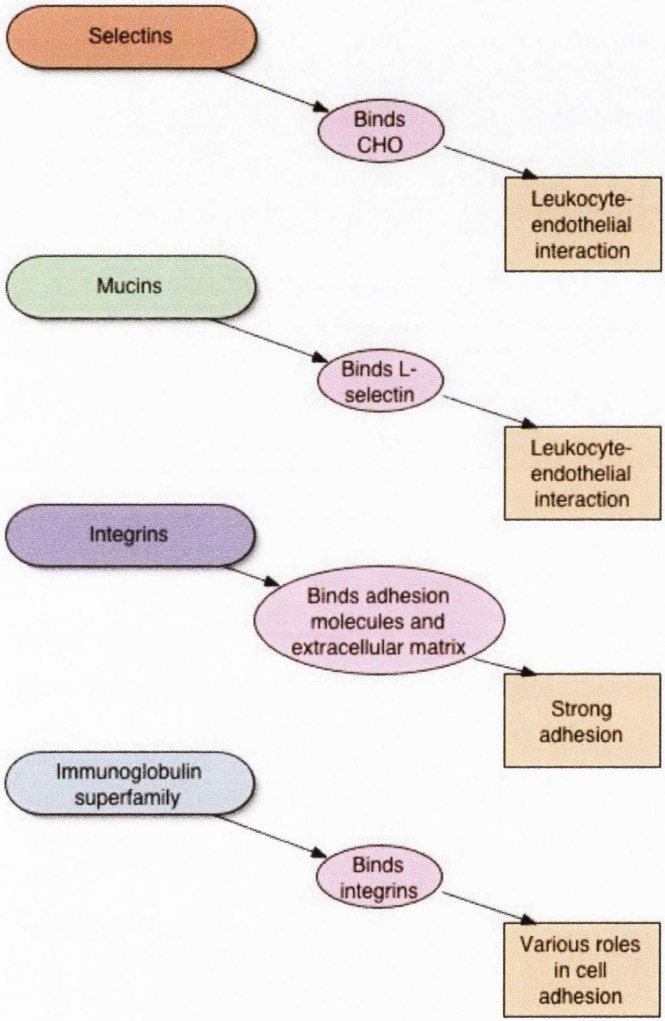


Fig. 3.1. The roles of molecules involved in leukocyte trafficking. CHO, carbohydrate.

The extravasation of leukocytes from the blood vessels to sites of infection or injury within tissues takes place in four stages. After the initial injury or insult the first chemical mediators to be released include CT4B, C5a and histamine. Within minutes of exposure P-selectin molecules begin to appear on the surface on the endothelial cells. Further mediators such as TNFα and lipopolysaccharide (LPS) stimulate expression on the endothelial cell surface of E-selectin for up to a few hours after the initial injury. Both P and E selectin appear to possess similar recognition sites allowing initial binding to take place (Erbe *et al.,* 1993). The expression of these molecules allow for weak reversible interactions to occur between the leukocytes and the endothelial cells. The reversible nature of the binding allows for tethering and rolling of the leukocytes along the blood, vessels, which in turn, aids the second step. As the rolling/tethering stage proceeds the leukocyte interacts with the integrins LFA-1 and CR3 and ICAM, which is induced by TNFα. Normally the binding between the leukocyte and the endothelium is still relatively weak at this stage but chemoattractants, such as IL-8, which is the main chemokine involved in initiating the adhesion and extravasation process (Taub *et al.,* 1996), cause a conformational change in the structure of both LFA-1 and CR3 allowing firm adhesion and arresting rolling. The leukocytes further adhere through LFA-1 and CR3 and then with the involvement of CD31, which is present at the intracellular junctions between the endothelial cells, enables the leukocytes to squeeze between the endothelial cells. Proteolytic enzymes can assist penetration of the basement membrane by breaking down the proteins of the membrane. This stage of the extravasation process is referred to as diapedesis and is followed by the continual migration of leukocytes through the tissue, via either a pore or through the intracellular junction, in response to a chemokine gradient bound to GAGs and or the Duffy receptor (Middleton *et al.,* 2002). See Fig. 3.2 for a graphical representation of the process.

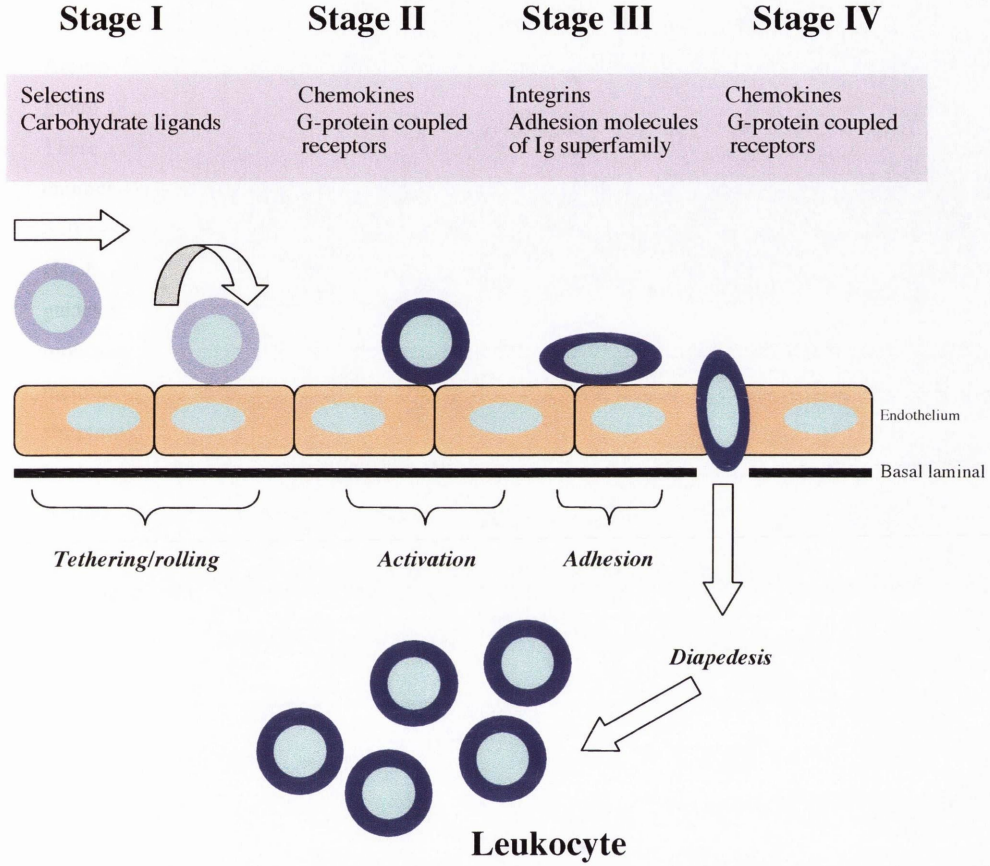


Fig. 3.2. The stages involved in leukocyte migration.

3.3 Amino Sugars

Amino sugars are aldoses or ketoses which have a hydroxyl group replaced by an amino group at any position other than the anomeric carbon and are commonly acetylated. These substances are named after the sugars from which they are derived by use of the enumerated prefix 'aminodeoxy'. 2-Amino-2-deoxy-D-glucose (D-glucosamine, Fig. 3.3) is abundant in nature, appearing in particular in the polysaccharide chitin (Wang & Hsieh 2002). Several other 2-amino-2-deoxy sugars also occur, 2-amino-2-deoxy-D-galactose (D-galactosamine) being one of the more common, since it is a constituent monosaccharide unit of dermantan and chondroitin sulphate, polysaccharides found in mammalian tissue and cartilage. Several clinically important antibiotics, e.g. streptomycin, erythromycin and gentamicin, have amino sugars as constituents and at least one amino sugar, nojirimycin, itself shows anti-bacterial activity (Ishida *et al.*, 1967) Amino sugars are essential for making glycoconjugates - macromolecules containing covalently bound oligosaccharide chains. Glycoconjugates include glycoproteins and glycolipids.

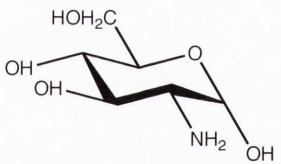


Fig. 3.3 General structure of an amino sugar. The sugar shown is 2-amino-2-deoxy-D-glucose (D-glucosamine), which is one of the simplest amino sugars.

3.3.1 Amino sugar metabolism and catabolism

Glucose is the metabolic precursor for all sugars not in the diet. The glycolytic intermediate, fructose-6-phosphate (F6P) is the precursor for the amino sugars. The nitrogen comes from the amide group of glutamine in the following reaction (Fig. 3.4), which is catalyzed by glutamine:fructose-6-phosphate aminotransferase.

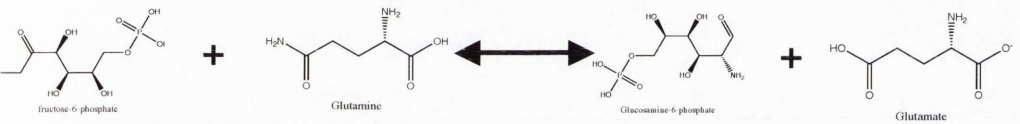
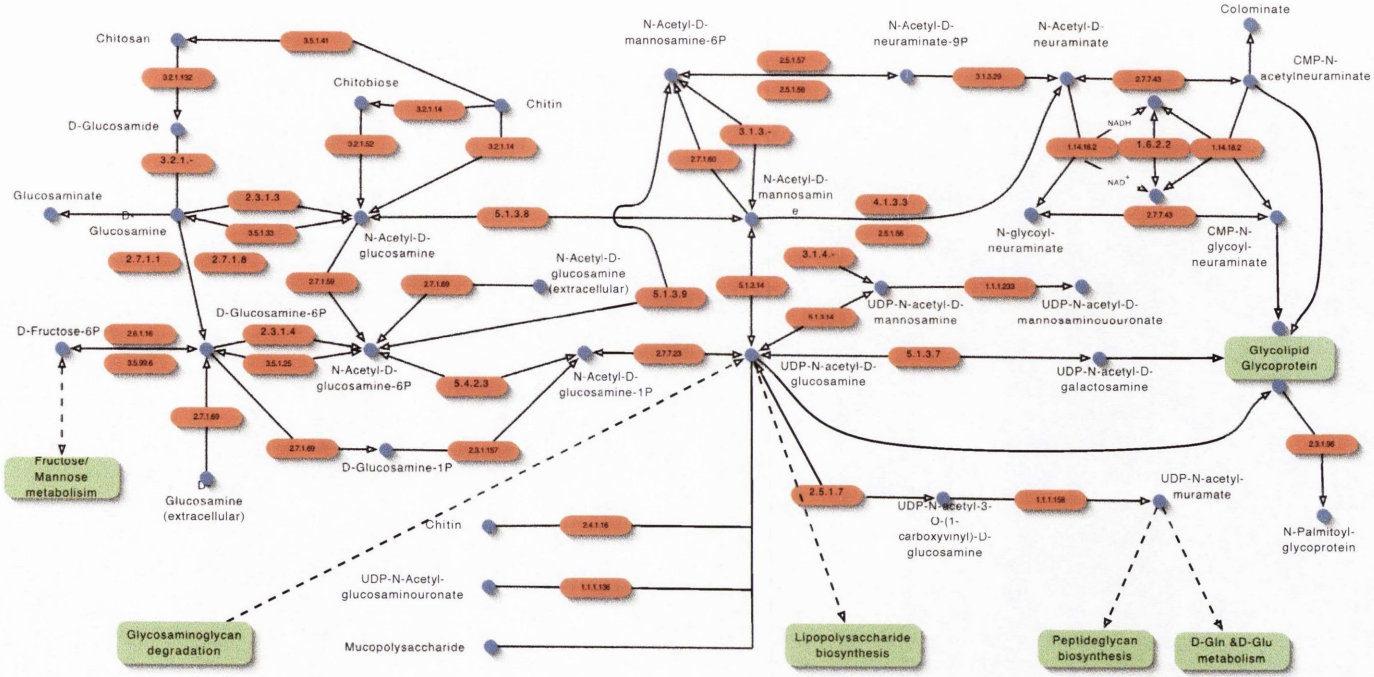


Fig. 3.4. Synthesis of glucosamine-6-phosphate

Further amino sugars can be synthesised from this starting point and can be metabolised for use within glycoproteins and glycolipids, See Fig. 3.5 for a map of amino sugar metabolism.

Fig. 3.5. Illustration of amino sugar metabolism and catabolism systems within mammals. Included are all intermediaries and enzymes. Enzymes are denoted by EC number and are identified in appendix 1. Information derived from the KEGG website. http://www.genome.jp/kegg/


Chitosan
3.5.1.41
3.2.1.132
Chitobiose
3.2.1.14
Chitin
D-Glucosamide
3.2.1.52
3.2.1.14
3.2.1.-
Glucosaminate
2.3.1.3
D-Glucosamine
3.5.1.33
N-Acetyl-D-glucosamine
5.1.3.8
2.7.1.1
2.7.1.8
2.7.1.59
2.7.1.69
N-Acetyl-D-glucosamine (extracellular)
5.1.3.9
D-Glucosamine-6P
D-Fructose-6P
2.6.1.16
3.5.99.6
2.3.1.4
3.5.1.25
N-Acetyl-D-glucosamine-6P
5.4.2.3
N-Acetyl-D-glucosamine-1P
2.7.7.23
2.7.1.69
2.3.1.157
D-Glucosamine-1P
Fructose/ Mannose metabolism
D-Glucosamine (extracellular)
N-Acetyl-D-mannosamine-6P
2.5.1.57
2.5.1.56
N-Acetyl-D-neuraminate-9P
3.1.3.29
N-Acetyl-D-neuraminate
2.7.7.43
Colominate
CMP-N-acetylneuraminate
NADH
NAD+
1.14.18.2
1.6.2.2
1.14.18.2
3.1.3.-
2.7.1.60
N-Acetyl-D-mannosamine
4.1.3.3
2.5.1.56
N-glycoyl-neuraminate
2.7.7.43
CMP-N-glycoyl-neuraminate
3.1.4.-
5.1.3.14
UDP-N-acetyl-D-mannosamine
1.1.1.233
UDP-N-acetyl-D-mannosaminououronate
5.1.3.14
UDP-N-acetyl-D-glucosamine
5.1.3.7
UDP-N-acetyl-D-galactosamine
Glycolipid Glycoprotein
2.3.1.96
N-Palmitoyl-glycoprotein
2.5.1.7
UDP-N-acetyl-3-O-(1-carboxyvinyl)-D-glucosamine
1.1.1.158
UDP-N-acetyl-muramate
Chitin
2.4.1.16
UDP-N-Acetyl-glucosaminouronate
1.1.1.136
Mucopolysaccharide
Glycosaminoglycan degradation
Lipopolysaccharide biosynthesis
Peptideglycan biosynthesis
D-Gln &D-Glu metabolism

3.3.2 Roles of amino sugars

The functional roles of amino sugars are as varied as they are numerous. Cell recognition predominates, as most ligands, such as the Lewis^x trisaccharide, consist of complex arrangements of amino sugars such as *N*-acetylgalactosamine combined with other carbohydrate moieties (Azurmendi *et al.*, 2002). Galactosamine has been shown to be the major carbohydrate constituent in synovial fluid (Swan & Radin 1972). The *N*-acetyl form of glucosamine is abundant in the oceans and, as such, is metabolised primarily by numerous marine bacteria, isolates of which have been shown to possess a phosphophoenolpyruvate:NAG phosphotransferase system. These organisms do not rely on *N*-acetyl-glucosamine alone and can utilise other sugars such as glucosamine, glucose, mannose and fructose via the same system (Riemann & Azam 2002). The cell walls of bacteria contain significant levels of amino sugars, for example in gram negative bacteria from 2-20% of the membrane isolated from a marine *Pseudomonas* species consisted of hexosamine with a significant portion being glucosamine (Martin & Macleod 1971). Similarly *Clostridium botulinum, Clostridium perfringens* and *E. coli* contained glucosamine and galactosamine in their cell membrane, which may aid in the ability of these bacteria to adhere to a host. (Fugate *et al.*, 1971). Of the recent roles elucidated for amino sugars, one of interest is a role for sialic acid metabolism in apoptosis, as *N*-acetyl-mannosamine analogs modulate apoptosis in two ways, the first is an indirect mechanism via hydroxyl group effects and the second is through effects mediated by the *N*-acetyl group (Kim *et al.*, 2004).

3.4 VAP-1/SSAO Story

Originally thought to be simply another vascular adhesion protein involved in mediating the migration of lymphocytes from blood vessels into tissue, see Fig 1.15 for adhesion scheme, VAP-1 was identified in 1992 (Salmi & Jalkanen 1992). The molecule itself was identified in several normal tissues and is up regulated during an inflammatory response. This suggested a role in extravasation, which was confirmed when an antibody directed against VAP-1 was shown to inhibit lymphocyte binding (Salmi *et al.*, 1993). Analysis

of the protein showed it to be a heavily glycosylated dimer with a molecular weight of 180kDa. Differences appeared in the post-translational modifications of the protein when comparing cultured endothelial cells with endothelial cells removed from tonsil. The cultured cell VAP-1 did not possess the abundant glycosylation or the peripheral lymph node addressins and was not expressed on the surface of the cell (Salmi & Jalkanen 1995). Although limited by the differences found in cultured cells, functional studies on the role of VAP-1 continued and VAP-1 on hepatic endothelium was found to bind T-cells and exhibited a level of specificity as only anti-VAP-1 antibodies prevented the interaction. Antibodies against several other common adhesion molecules were ineffective in preventing the adhesion of the T-cells (McNab *et al* 1996). The T-cell binding nature of VAP-1 was shown to play an important role in the recruitment and infiltration of hepatic tumours (Yoong *et al* 1998). VAP-1 also appears to be important in mediating lymphocyte binding to inflamed skin, in concert with peripheral lymph node addressin (Arvilommi *et al.,* 1996). Similarly VAP-1 has been implicated in the binding of mucosal effector cells to synovium in a leukocyte-subtype selective manner (Salmi *et al* 1997). The distinct pattern of expression in these specific tissues, along with expression noted in gut under inflammatory conditions, suggested a highly-regulated controlled infrastructure. Regulators, under certain conditions, included chemokines, such as IL-1, IL-4 , TNF α, INF γ whereas other known mediators of inflammatory responses were found to show no such regulation on VAP-1 (Arvilommi *et al.,* 1997). The mechanism of action for the binding promoted by VAP-1 is similar to that of other cell-adhesion proteins, whereby cell to cell interaction is facilitated by the sialic residues present on the external surface of the protein. Once desialyated the adhesion function of VAP-1 is essentially lost (Salmi & Jalkanen 1996). The target ligands for VAP-1 adhesion are as yet unknown but L-selectin, P-selectin glycoprotein-ligand 1 and α-4 intergrins have been discounted, as VAP-1 mediated binding is independent of the aforementioned ligands in the recognition of subpopulations of mononuclear cells (Salmi *et al.,* 1997).

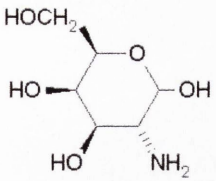
Attempts at cloning resulted in the production of a mouse form of VAP-1, which when characterised was found to share significant identity with the copper-containing amine oxidase family of enzymes (Bono *et al.,* 1998a). Later cloning of the human form of

VAP-1 also showed copper amine oxidase activity and further structural information revealed that VAP-1 is a type II transmembrane protein with a single transmembrane domain located at the NH_2-terminal end of the molecule and six potential *N*-glycosylation sites in the extracellular domain, essentially confirming that VAP-1 is a copper-containing amine oxidase similar to SSAO and is now considered to be an SSAO (Smith *et al.*, 1998).

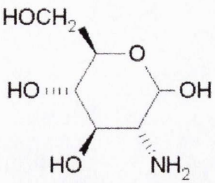
Cloning of the protein also revealed useful information regarding the genetic organisation of VAP-1 from mouse. The gene itself is approximately 14.4 kb in length containing 4 exons and 3 introns but, interestingly, different tissues had varying transcription initiation sites, possibly allowing different regulation within the different tissues (Bono *et al.*, 1998b). Demonstration of the adhesion ability of VAP-1 was again proved when a non-adhering endothelial cell line was transected with VAP-1 cDNA, this cell line was then conferred with an adhesion function, possibly activated through CD44 (Salmi *et al.*, 2000). Further studies have shown the numerous roles VAP-1 plays in modulating leukocyte responses during inflammation (Jaakkola *et al.*, 2000) and disease states, such as an increase in its activity in chronic liver disease (Kurkijarvi *et al.*, 2000), where VAP-1 aids in the recruitment of lymphocytes to the liver (Lalor *et al.*, 2002). Intense expression of VAP-1 is found in treatment-resistant lyme arthritis (Akin *et al.*, 2001) and it has been shown to aid the binding of tumour infiltrating lymphocytes (TIL) to tumor endothelium thereby increasing the effectiveness of the TILs (Irjala *et al.*, 2001). The effectiveness of the role VAP-1 has under these conditions can be emphasised in skin malignant melanomas. In the intratumoral microvessels of human skin melanoma there is a loss of VAP-1 expression, which did not correlate with the density of T-cells. Patients who exhibited lower VAP-1 expression also had a lower survival rate than patients who had high VAP-1 expression (Forster-Horvath *et al.*, 2004). Furthers studies have increased our understanding of the role of VAP-1in inflammation, and there is now evidence of an increase in expression under, certain conditions, in areas of the lung that are not normally associated with its presence, allowing for cell infiltration (Singh *et al.*, 2003). Many of these changes reported in VAP-1 mirror changes previously identified in SSAO under similar conditions, See section 1.3.7.

3.5 Aims

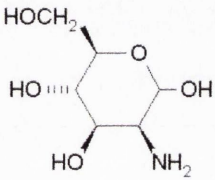
With the adhesion role of VAP-1 established along with confirmation that it is an SSAO, it became of interest to determine if the enzymatic activity is involved, in some way, in the adhesion mechanism. As previously discussed, amino sugars are prevalent within the complex carbohydrate moieties found throughout cell adhesion and recognition, and they also possess an amino group which may be recognized by an amine oxidising enzyme such as SSAO.



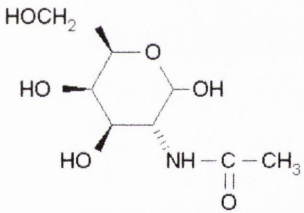
Galactosamine



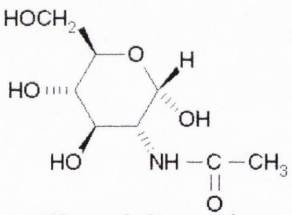
Glucosamine



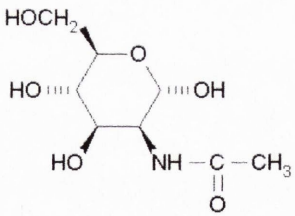
Mannosamine



***N*-acetyl galactosamine**



***N*-acetyl glucosamine**



***N*-acetyl mannosamine**

Fig. 3.6. The amino hexoses used in this study.

3.6 Methods (Not included in chapter 2)

3.6.1 Enzyme assays

Oxidation of benzylamine, methylamine by SSAO was determined as outlined in sections 2.4.1 and 2.4.2. Determination of hydrogen peroxide was determined as described in section 2.4.3

3.6.2 Kinetic Analysis of the inhibition of SSAO by Galactosamine

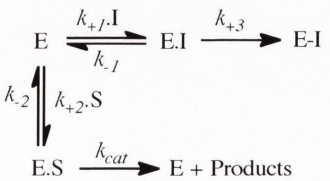
Kinetic analysis of the time-dependent inhibition of SSAO by galactosamine during the amine-oxidation reaction was achieved by fitting the reaction progress-curve data in the presence of the amine substrate (Fig 3.5) according to the equation (see Tipton, 1996):

$$[P_t] = [P_\infty](1 - e^{-k'.t}) \qquad (1)$$

where $[P_t]$ and $[P_\infty]$ are the product concentrations at any time, t, and at complete cessation of the reaction, respectively, and k' is the apparent first-order rate constant for the decline in enzyme activity. Square brackets are used to denote concentrations.
The substrate-concentration dependence of the apparent first-order rate constants at each amino-sugar concentration was fitted to the relationship,

$$k' = \frac{k_{+3}}{1 + \frac{K_i}{[I]}\left(1 + \frac{[S]}{K_m}\right)} \qquad (2)$$

which describes the situation where substrate and inhibitor both compete for non-covalent binding to the enzyme, according to the system:



(3)

in which K_m is the Michaelis constant in the absence of inhibitor ($\{k_{-2} + k_{cat}\}/k_{+2}$) and, assuming $k_{+3} << k_{-1}$ (see Tipton, 1996), $K_I = k_{-I}/k_{+I}$.

The data were also fitted to the equation

$$k' = \frac{k_{app}}{1 + \frac{[S]}{K_m}} \qquad (4)$$

which describes the situation where the inhibitor does not form an initial non-covalent complex with the enzyme

$$\begin{array}{ccc} E & \xrightarrow{k.I} & E\text{-}I \\ {\scriptstyle k_{-I}}\uparrow\downarrow{\scriptstyle k_{+I}.S} & & \\ E.S & \xrightarrow{k_{cat}} & E + \text{Products} \end{array} \qquad (5)$$

where determination of the *pseudo* first-order $k_{app} = k$ [I], was also attempted for comparative purposes.

K_I, k_{app} and k_{+3} values were determined using the commercial software program MacCurveFit 1.5. (Kevin Raner Software, Australia). Data are displayed as a double-reciprocal representation of Equation (3) for illustrative purposes only.

3.7 Results

3.7.1 The effect of physiological relevant amino hexoses on the oxidative deamination of benzylamine by semicarbazide-sensitive amine oxidase (SSAO)

Time-dependent inhibition of the oxidative deamination of benzylamine by bovine-plasma semicarbazide sensitive amine oxidase was observed in the presence of the amino sugars mannosamine, glucosamine and galactosamine, each at a concentration of 1mM. The observed inhibition was time dependent and the inhibition by galactosamine was the most effective (Fig. 3.7.1). The parent sugars and their *N*-acetyl derivatives had no effect on the activity of SSAO under these conditions.

3.7.2 The effect of the amino hexoses, galactosamine and mannosamine on the oxidative deamination of benzylamine by semicarbazide sensitive amine oxidase (SSAO)

When present in the assay mixture a range of galactosamine concentrations (up to 10 mM) inhibited plasma SSAO in a time- and concentration-dependent manner (Fig. 3.7.2). Inhibition of SSAO by 10 mM galactosamine was found to be fully reversible after dilution experiments where 85% of activity was recovered, compared to the uninhibited control (Fig. 3.7.3). Using an alternative SSAO substrate, methylamine, inhibition of SSAO activity was also observed in the presence of galactosamine, which was also dependent on time and galactosamine concentration (Fig. 3.7.4). Bovine lung microsomal SSAO, was investigated in a similar way, as an example of the membrane- bound form of the enzyme. As shown in Fig. 3.7.5, it was also inhibited by galactosamine in the time- and concentration-dependent manner The K_i for the inhibition of bovine plasma SSAO by galactosamine was calculated by fitting the reaction-progress curves to Eqs. (1) and (2) (see Section 3.6.1) and was found to be 3.9 ± 1.6 mM. The apparent first-order rate constant (k_{+3}) for the time-dependent inhibition was 0.029 ± 0.004 min^{-1} (see Figs 3.7.6 and 3.7.7).

Determination of hydrogen peroxide formation when SSAO was incubated with the amino sugars, in the absence of benzylamine or methylamine, for periods up to 2 hours indicated that they were not substrates for the enzyme. Time and concentration dependent inhibition was also observed in the presence of mannosamine, albeit with a lower potency, see (Fig.3.7.8).

3.7.3 Effects of the products from the oxidative deamination of benzylamine by SSAO, on the inhibition of SSAO by galactosamine.

No significant inhibition was observed when bovine-plasma semicarbazide sensitive amine oxidase was incubated, at 37 °C, with 5 mM galactosamine for times up to 100 min (Fig. 3.7.10). Similarly, preincubation of the enzyme with 5 mM galactosamine in the presence of either 1 mM ammonium chloride or 1 mM benzaldehyde before assaying the activity towards benzylamine did not result in time-dependent inhibition. However, as shown in Fig. 3.7.9, when SSAO was preincubated with 5 mM galactosamine in the presence of 1 mM hydrogen peroxide for various times before adding substrate it resulted in time-independent inhibition. No time-dependent inhibition occurred with preincubation of the enzyme with hydrogen peroxide alone (Fig. 3.7.10).

3.7.4 Removal of hydrogen peroxide by catalase abolishes the time-dependent inhibition of SSAO activity by galactosamine.

As shown in Fig. 3.7.11 no significant inhibition of the enzyme occurred when catalase (1.5 units) was present in the preincubation mixture containing both hydrogen peroxide (1mM) and galactosamine (5 mM). When the time course of benzylamine oxidation was followed in the presence of galactosamine (5mM), the inclusion of catalase reduced but did not completely eliminate the galactosamine-induced inhibition (Fig. 3.7.12). However the addition of catalase after 250 min, when the inhibition was essentially complete, to the reaction had no significant effect on the inhibition, (see Fig. 3.7.13).

3.7.5 Determination of any possible synergism between galactosamine, hydrogen peroxide and benzylamine.

No significant inhibition of the initial rate of benzylamine oxidation was observed after hydrogen peroxide (1 mM) and galactosamine (3 mM) had been preincubated for 24 h before addition of the enzyme and substrate (Fig. 3.7.14). Similarly, a 24 h preincubation of benzylamine (5 mM) and 3 mM galactosamine did not result in the formation of an inhibitory species (Fig 3.7.15).

3.7.6 The effect of three structurally non-physiological amino hexose analogues on the oxidative deamination of benzylamine by SSAO.

Three non-commercially available amino sugars were kindly provided by Dr. Paul Murphy (Conway Institute, University College Dublin). Their structures are shown in Figs. 3.7.16 - 3.7.18. The effects of amino hexose DD6B (tested at 1 mM and 100 μM) on bovine plasma SSAO showed significant inhibition when added to the benzylamine-assay mixture, but this did not increase with time,as shown in Fig. 3.7.16. The amino hexose DD6D (also tested at 1 mM and 100 μM) behaved similarly, although the inhibitory potency was lower than that of DD6B (Fig. 3.7.17). The amino hexose DD6E had no significant inhibitory effect on the oxidative deamination of benzylamine by SSAO (Fig. 3.7.18).

3.7.7 Studies on the effects of other compounds with some structural features resembling those of the amino hexoses

Serinol (1 nM–10 mM), which has partial structural similarity to amino sugars, had no inhibitory effects on benzylamine oxidation by bovine plasma SSAO (Fig. 3.7.19). Pyrroline-5-carboxylic acid (1μM - 500μM), a larger molecule than serinol but also similar in structure to the amino sugars, showed concentration dependent inhibition of

benzylamine oxidation by bovine plasma SSAO, however, no time-dependent inhibition was observed (Fig 3.7.20).

3.7.8 Summary of the compounds in this study and their effect (if any) on the oxidative deamination of amine substrates by SSAO.

Compound	Range	Level of inhibition	Time dependent
Galactosamine	0.3-10 mM	+++	Yes
Mannosamine	1 mM-10 mM	+	Yes
Glucosamine	1 mM	++	Yes
N-Acetylgalactosamine	1 mM-25 mM	N.D.	-
N-Acetylmannosamine	1 mM-25 mM	N.D.	-
N-Acetylglucosamine	1 mM-25 mM	N.D.	-
Glucose	1 mM-25 mM	N.D.	-
Galactose	1 mM-25 mM	N.D.	-
Mannose	1 mM-25 mM	N.D.	-
DD6B	100 μM-1 mM	+++	No
DD6D	100 μM-1 mM	+	No
DD6E	100 μM-1 mM	N.D.	-
Serinol	1 nM-10 mM	N.D.	-
Pyrroline-5-carboxylic acid	1 μM- 500 μM	+++	No

Table 3.7.1 Compounds used in this study and their effects on SSAO activity when present during an assay with benzylamine as substrate. N.D., not detected.

Mixture	Range	Preincubated
Galactosamine	0.3 mM-10 mM	Up to 24 h
SSAO + Galactosamine	0.3 mM-10 mM	Up to 5 h
Galactosamine + H_2O_2	3 mM / 1 mM	Up to 24 h
SSAO + H_2O_2	0.012 mg/ml / 1 mM	Up to 5 h
Galactosamine + NH_4^+	5 mM /1 mM	Up to 5 h
SSAO + Galactosamine + NH_4^+	012 mg/ml / 5 mM / 1 mM	Up to 5 h
Galactosamine + Benzaldehyde	5 mM /1 mM	Up to 5 h
SSAO + Galactosamine + Benzaldehyde	012 mg/ml / 5 mM / 1 mM	Up to 5 h
Galactosamine + H_2O_2 + Catalase	3 mM /1 mM / 1.5 u	Up to 5 h
SSAO + Galactosamine + H_2O_2 + Catalase	0.012 mg/ml /3 mM /1 mM / 1.5 u	Up to 5 h

Table 3.7.2 Preincubations before addition to the standard assay.

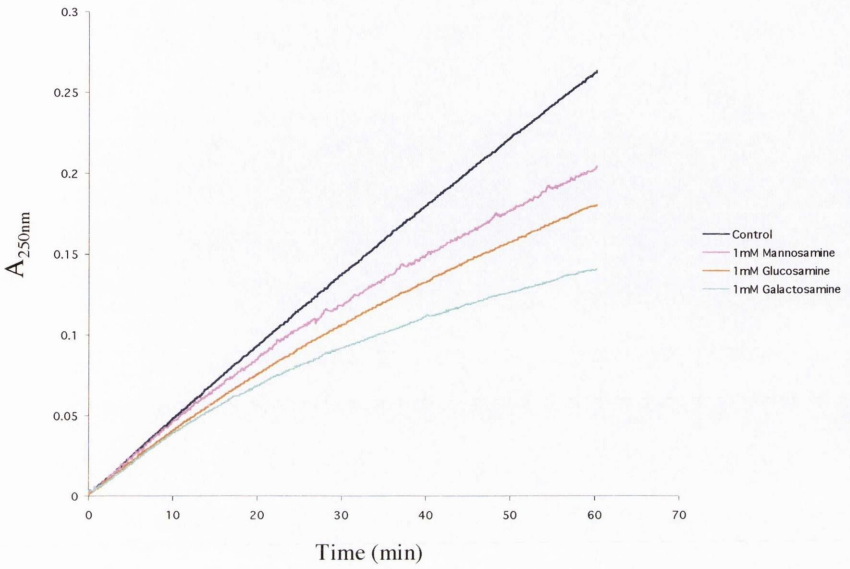


Fig. 3.7.1 Time course of the oxidation of benzylamine by bovine plasma SSAO (Sigma) in the presence of several different amino sugars.

The enzyme (protein concentration 0.024 mg/ml) was preincubated in potassium phosphate buffer (50 mM, pH 7.2) for 5 min at 37°C. The reaction was started by the addition of 5 mM benzylamine. The production of benzaldehyde was captured at 250 nm, using a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Representative progress curve of at least 4 replicates is shown. Errors bars were omitted for clarity.

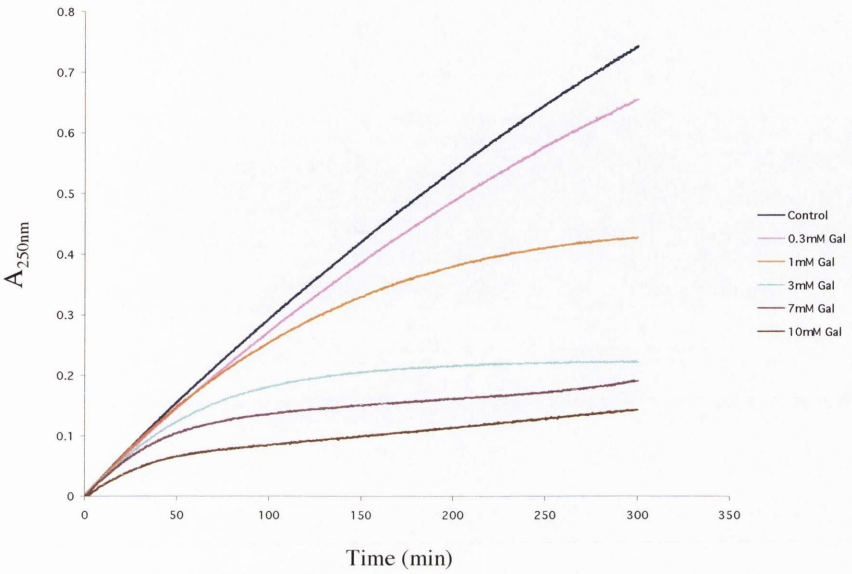


Fig. 3.7.2 Time course of the oxidation of benzylamine by bovine plasma (Sigma) SSAO in the presence of galactosamine.

Bovine plasma amine oxidase at a concentration of 0.012 mg/ml was preincubated in potassium phosphate buffer (50 mM, pH 7.2) for 5 min at 37°C. The reaction was started by the addition of 5 mM benzylamine. The production of benzaldehyde was determined at 250 nm, using a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each curve is a representative progress curve of at least 6 replicates. Errors bars were omitted for clarity.

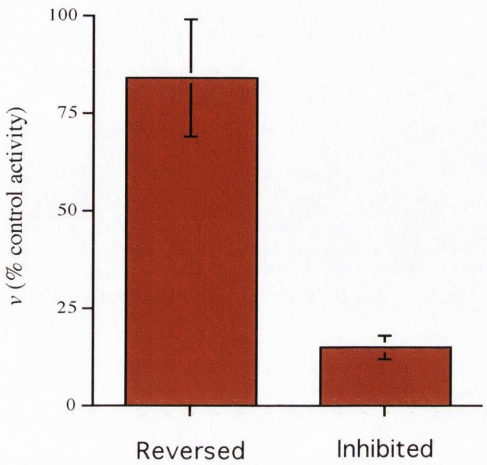


Fig. 3.7.3. Reversibility of the inhibition of plasma SSAO (Sigma) by galactosamine.

Bovine plasma amine oxidase at a protein concentration of 1.2 mg/ml, was incubated with 10 mM galactosamine in the presence of 5 mM benzylamine until inhibition was essentially complete, at approximately 2 hours. Aliquots of 10 μl were then removed into the standard benzylamine assay mixture to give a final protein concentration of 0.012mg/ml, for activity determinations. Each point is the mean value ± S.E.M. from three separate experiments.

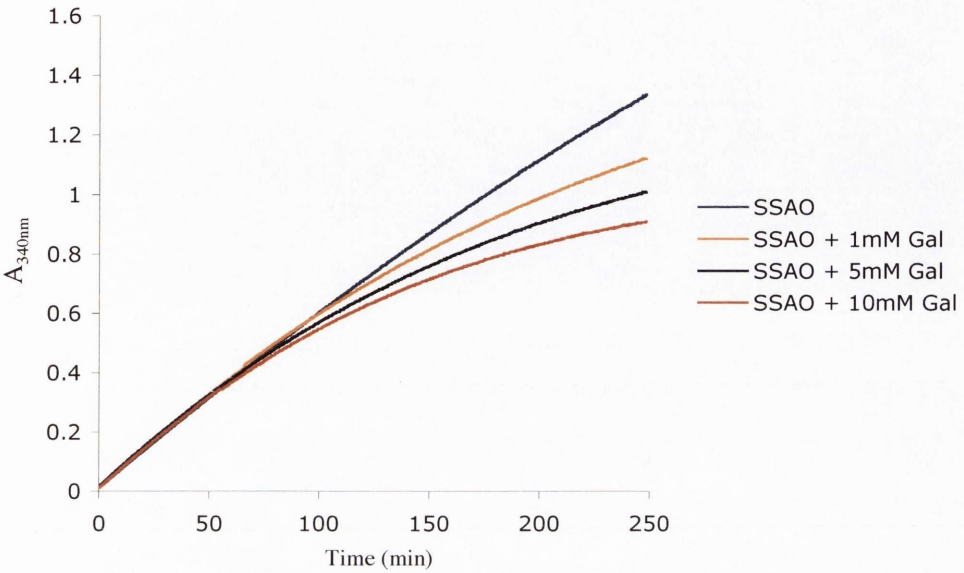


Fig. 3.7.4. Time courses of the oxidation of methylamine by bovine plasma SSAO (Sigma) in the presence of galactosamine.

Protein (0.024 mg/ml) was preincubated in potassium phosphate buffer (50 mM, pH 7.2) containing 0.01 unit of FDH and 200 μM NAD for 5 min at 37 ^{o}C. The reaction was started by the addition of 15 mM methylamine and the production of formaldehyde was determined by the rate of absorbance at 340 nm, using a Varian Cary 300 bio spectrophotometer (Tipton *et al*, 2000) Each curve is a representative progress curve of at least 6 replicates. Errors bars were omitted for clarity.

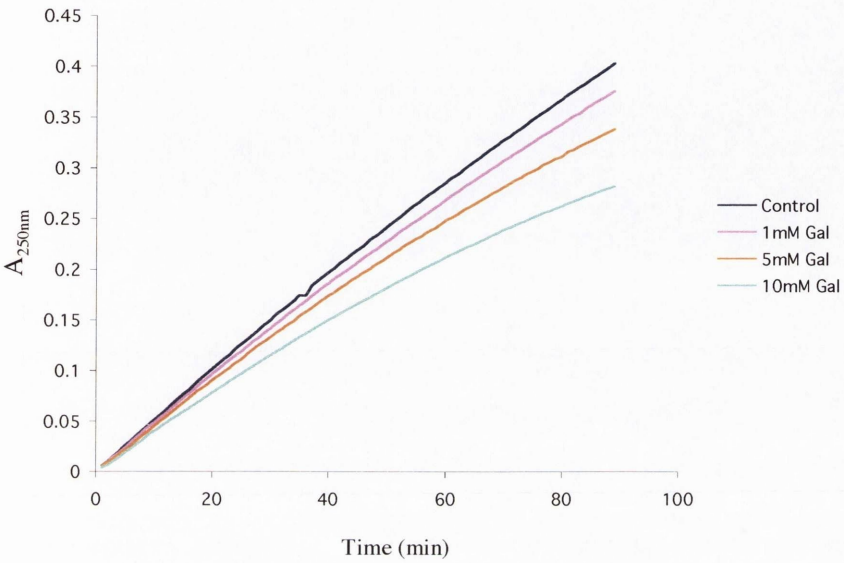


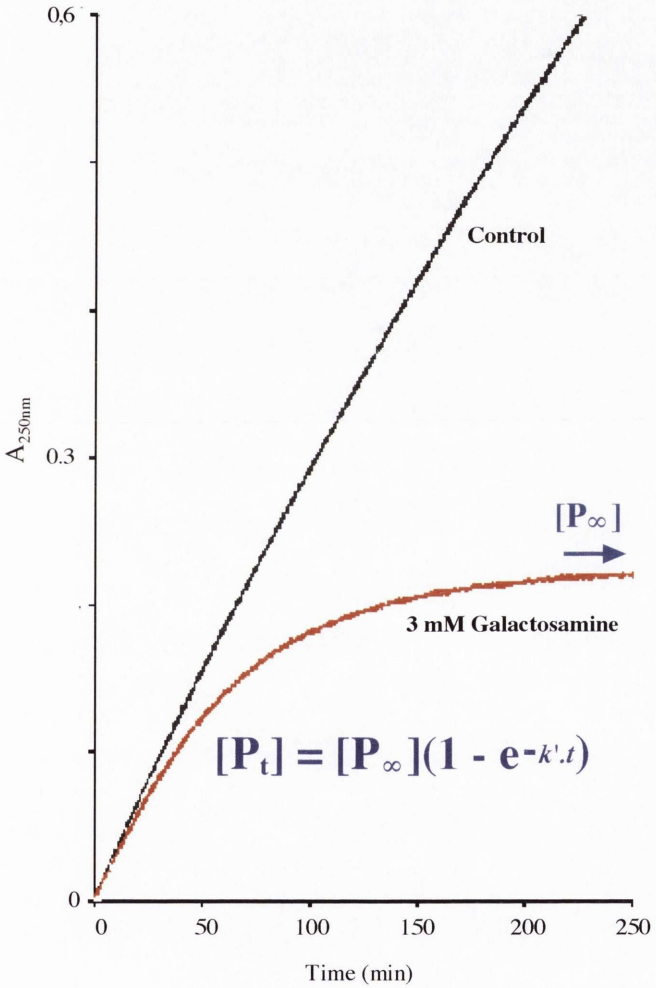
Fig. 3.7.5 Time courses of the oxidation of benzylamine by bovine lung membrane bound SSAO (microsomes) in the presence of galactosamine.

The enzyme, at a protein concentration of 0.5 mg/ml, was preincubated in potassium phosphate buffer (50 mM, pH 7.2) for 5 min at 37 ^{O}C. The reaction was started by the addition of 5 mM benzylamine. The production of benzaldehyde .was determined at 250 nm, using a Perkin Elmer spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each curve is a representative progress curve of at least 6 replicates. Errors bars were omitted for clarity.

Fig. 3.7.6 Kinetic analysis of the time-dependent inhibition of bovine plasma SSAO by galactosamine in the reaction was achieved by fitting the reaction progress-curves data in the presence of the 5 mM benzylamine (Fig 3.5) according to the equation (see Tipton, 1996)

$$[P_t] = [P_\infty](1 - e^{-k'.t}) \qquad (1)$$

where $[P_t]$ and $[P_\infty]$ are the product concentrations at any time, t, and at complete cessation of the reaction, respectively, and k' is the apparent first-order rate constant for the decline in enzyme activity.


0,6
0.3
0
A_{250nm}
Control
$[P_\infty]$
3 mM Galactosamine
$[P_t] = [P_\infty](1 - e^{-k'.t})$
0
50
100
150
200
250
Time (min)

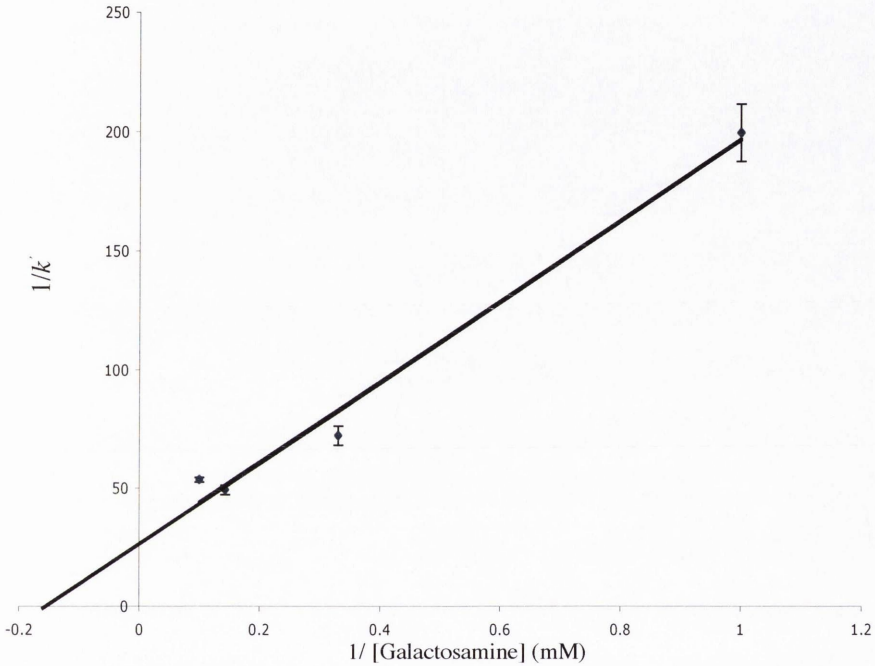


Fig. 3.7.7 Variation of the apparent first order rate constant for the time dependent inhibition of bovine plasma SSAO (Sigma) oxidizing 5 mM benzylamine in the presence of the amino sugar galactosamine. Data are displayed as a double-reciprocal plot for illustrative purposes only. Each point is the mean value ± S.E.M (n = 3).

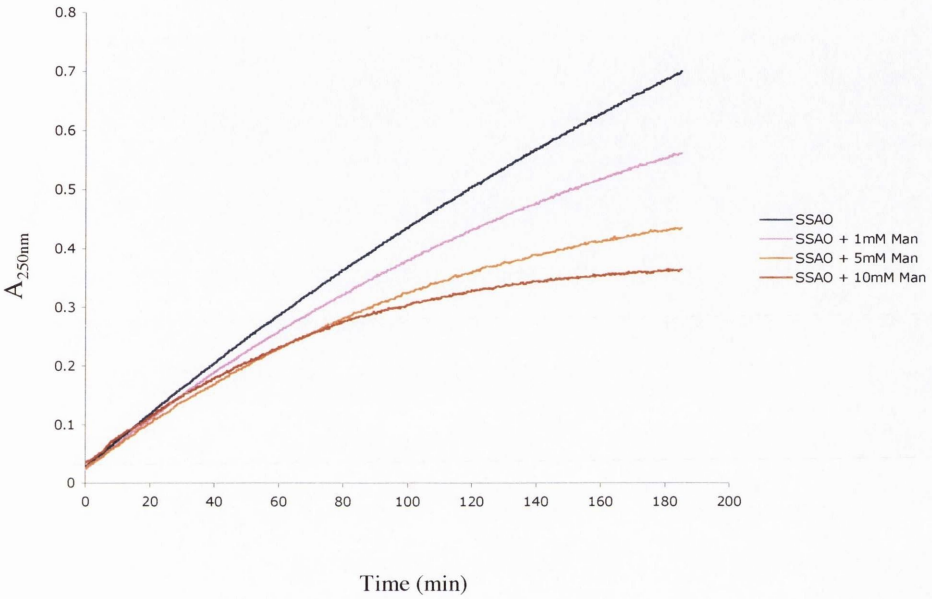


Fig. 3.7.8 Time courses of the oxidation of benzylamine by bovine plasma SSAO (Sigma) in the presence of mannosamine.

The enzyme, at a protein concentration of 0.012 mg/ml, was preincubated in potassium phosphate buffer (50 mM, pH 7.2) for 5 min at 37 ^{o}C. The reaction was started by the addition of 5 mM benzylamine. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each curve is a representative progress curve of at least 6 replicates. Errors bars were omitted for clarity.

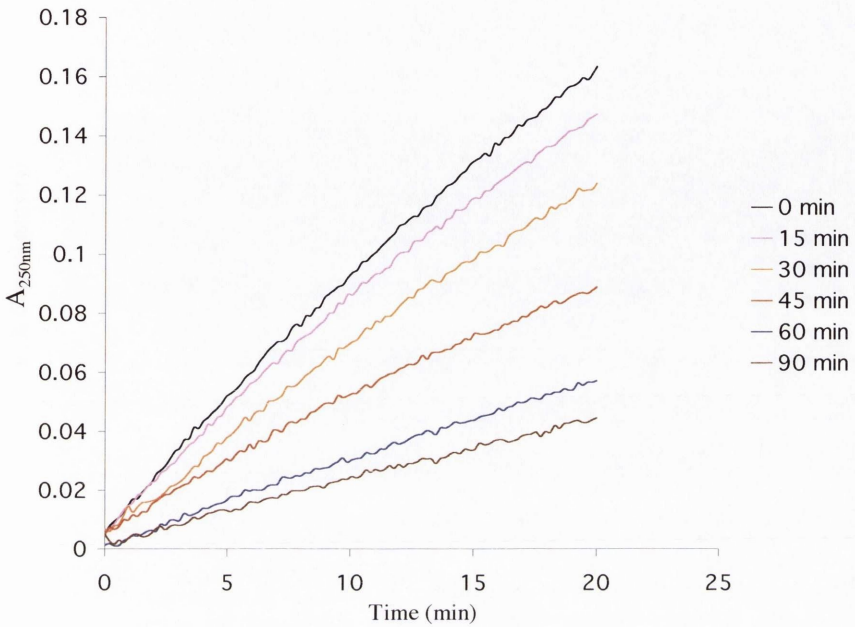


Fig. 3.7.9 The effect of preincubation of SSAO (Sigma) with galactosamine (5 mM) and H_2O_2 (5 mM) on the initial rates of benzylamine oxidation.

Bovine plasma SSAO (0.024 mg/ml) was incubated at 37 ^{O}C with the indicated concentrations of galactosamine and H_2O_2. The reaction was started at the indicated times by the addition of 5 mM benzylamine. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each curve is a representative progress curve of at least 3 replicates. Errors bars were omitted for clarity.

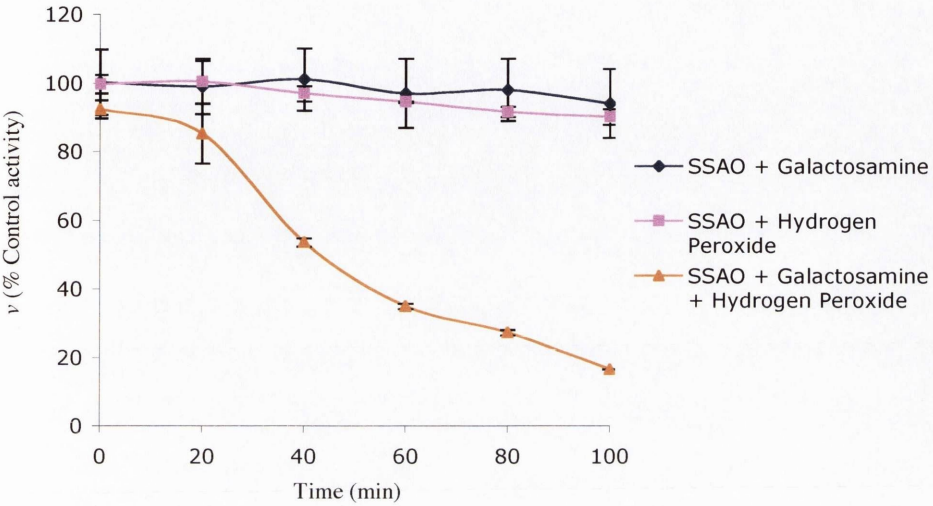


Fig. 3.7.10 The effects of preincubation of SSAO (Sigma) with galactosamine (5 mM) and H_2O_2 (1 mM) on the initial rates of benzylamine oxidation.

Bovine plasma SSAO (0.012 mg/ml) was incubated 37 ^{o}C with the indicated concentrations of galactosamine and H_2O_2. The reaction was started at the indicated times by the addition of 5 mM benzylamine. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each point is the mean value ± S.E.M. from three separate experiments.

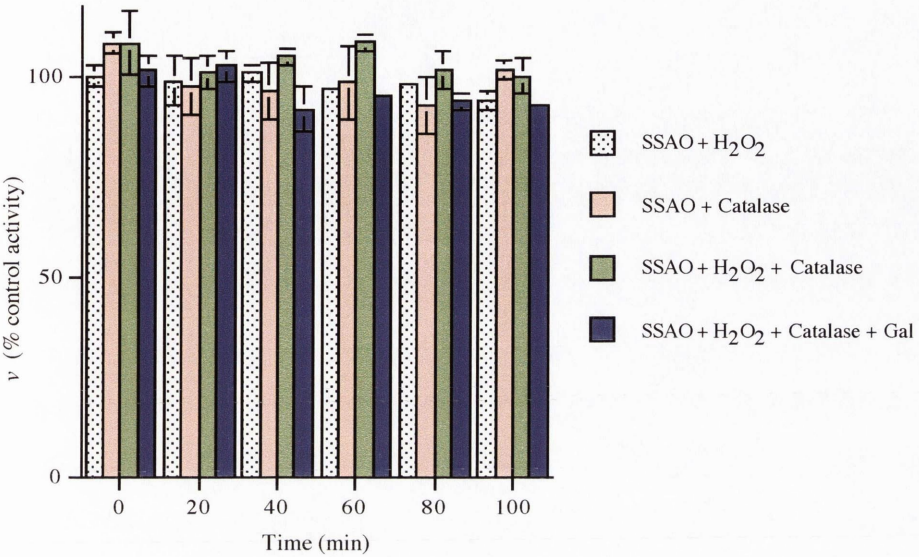


Fig. 3.7.11 The effect of preincubation of SSAO (Sigma) with galactosamine (Gal 5 mM), H_2O_2 (1 mM) and catalase (1.5 units) on the initial rates of benzylamine oxidation.

Bovine plasma SSAO (0.012 mg/ml) was incubated 37 ^{o}C with the indicated concentrations of galactosamine, H_2O_2 and catalase. The reaction was started at the indicated times by the addition of 5 mM benzylamine. The production of benzaldehyde was determined at 250 nm, using a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each value is the mean ± S.E.M. from six separate experiments.

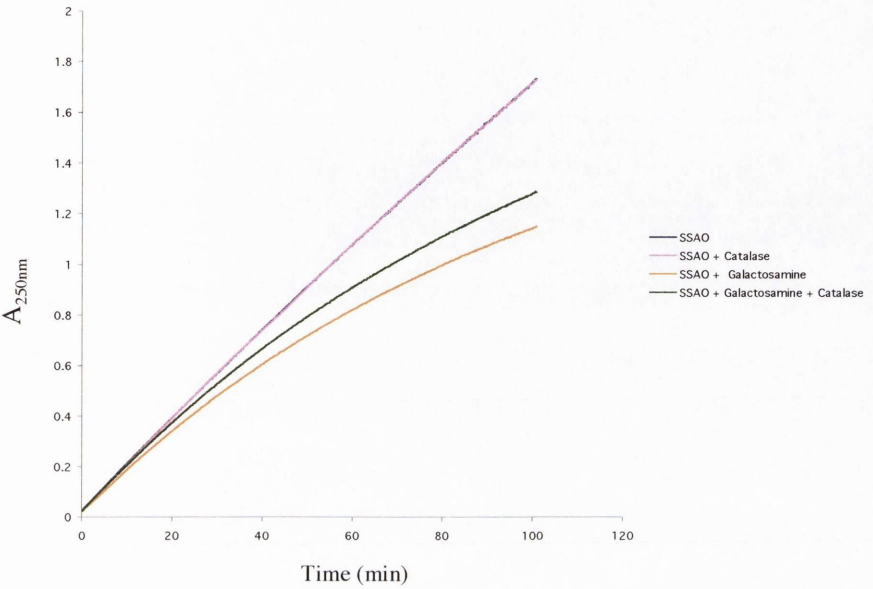


Fig. 3.7.12 Time courses of the oxidation of benzylamine by bovine plasma SSAO (Sigma) in the presence and absence of galactosamine (5 mM) and catalase (0.15 units).

Protein (0.012mg/ml) was preincubated in potassium phosphate buffer (50 mM, pH 7.2) with galactosamine and catalase at the stated concentrations for 5 min at 37°C. The reaction was started by the addition of 5 mM benzylamine. The production of benzaldehyde was determined at 250 nm, using a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each curve is a representative progress curve of at least 6 replicates. Errors bars were omitted for clarity.

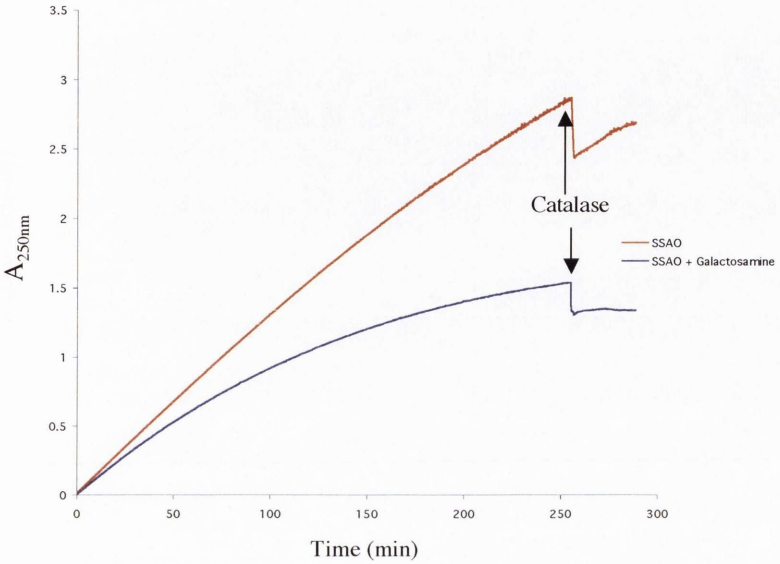


Fig. 3.7.13 Time course of the oxidation of benzylamine by bovine plasma SSAO (Sigma) in the presence and absence of galactosamine (5mM).

Protein (0.012mg/ml) was preincubated in potassium phosphate buffer (50 mM, pH 7.2) with galactosamine for 5 min at 37 ^{o}C. The reaction was started by the addition of 5mM benzylamine. The production of benzaldehyde was determined at 250 nm, using a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each curve is a representative progress curve of at least 6 replicates. Errors bars were omitted for clarity.

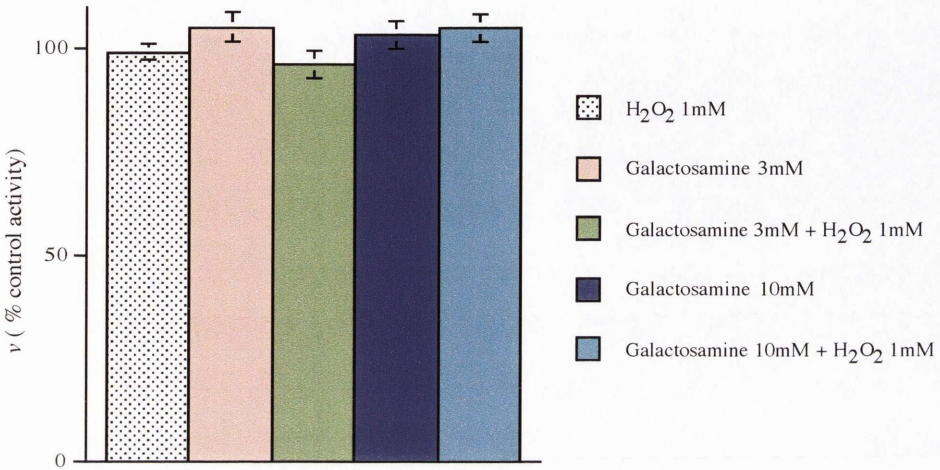


Fig. 3.7.14 The effect of a 24 hr incubation of H_2O_2 (1 mM) with galactosamine (3 mM and 10 mM) at 37^{O}C on the initial rates of benzylamine oxidation by SSAO.

Bovine plasma SSAO (0.012 mg/ml) was added to the preincubation mixture of H_2O_2 and galactosamine. The reaction was started at the indicated times by the addition of 5mM benzylamine after 5 min at 37 ^{O}C. was determined at 250 nm, using a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each value is the mean $\pm$ S.E.M. (n = 3).

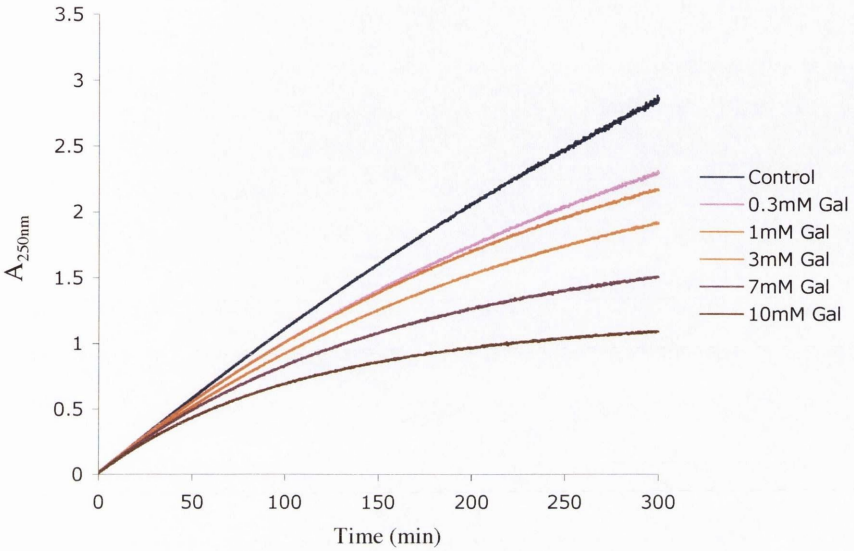
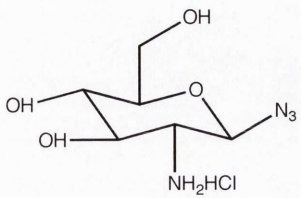


Fig. 3.7.15 The effect of a 24 h incubation of benzylamine (5 mM) with galactosamine at 37 ^{O}C on the initial rates of benzylamine oxidation by SSAO.

Bovine plasma SSAO (0.024 mg/ml) was added to the preincubation mixture of H_2O_2 and galactosamine at the stated concentrations. The reaction was started at the indicated times by the addition of 5 mM benzylamine after 5 min at 37^{O}C. was determined at 250 nm, using a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each point is the mean value ± S.E.M. from six separate experiments.

Fig. 3.7.16 The effect on the initial rate SSAO (partially purified) oxidative deamination of benzylamine by the amino sugar DD6B (see structure below) synthesized by Dr. Paul Murphy, Conway Institute, University College, Dublin.

The sugar (1 mM and 100 μM) was incubated for 5 min with 0.01mg/ml of protein at 37 ^{O}C. The reaction was initiated by the addition of 20 μM of ^{14}C-labelled benzylamine with a specific activity of 0.3 μCi/μmol. The reaction was terminated after 10 min by the addition of 100 μl of 2 M citric acid (Tipton *et al.*, 2000). Samples were the extracted in scintillant and counted on TriCarb 2100 scintillation spectrophotometer, as described in Section 2.4.4. Each value is the mean ± S.E.M. (n = 3)


OH
OH
O
N_3
OH
NH_2HCl

DD6B-2(+HCl)

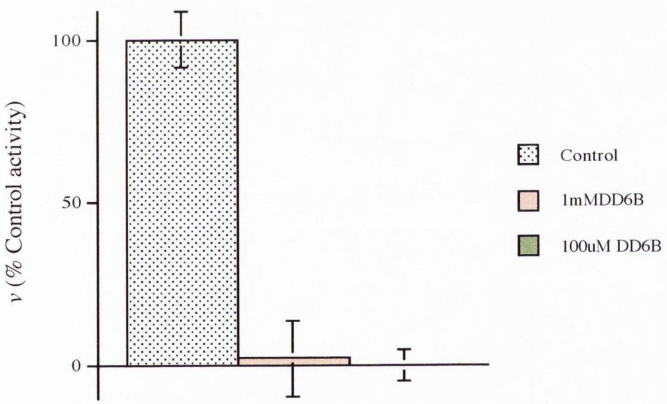
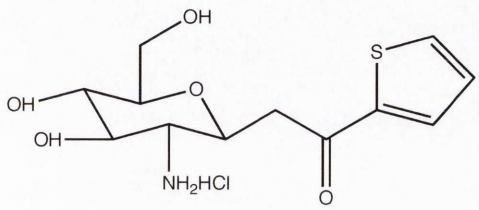

100
50
0
v (% Control activity)
Control
1mMDD6B
100uM DD6B

Fig. 3.7.17 The effect on the initial rate SSAO (partially purified) oxidative deamination of benzylamine by the amino sugar DD6D (see structure below) synthesized by Dr. Paul Murphy, Conway Institute, University College, Dublin.

The sugar (1 mM and 100 μM) was incubated for 5 min with 0.01mg/ml of protein at 37 ^{o}C. The reaction was initiated by the addition of 20 μM of ^{14}C-labelled benzylamine with a specific activity of 0.3 μCi/μmol. The reaction was terminated after 10 min by the addition of 100 μl of 2 M citric acid (Tipton *et al.*, 2000). Samples were the extracted in scintillant and counted on TriCarb 2100 scintillation spectrophotometer, as described in Section 2.4.4. Each value is the mean ± S.E.M. (n = 3)

DD6D-2(+HCl)


OH
S
O
OH
OH
NH_2HCl
O

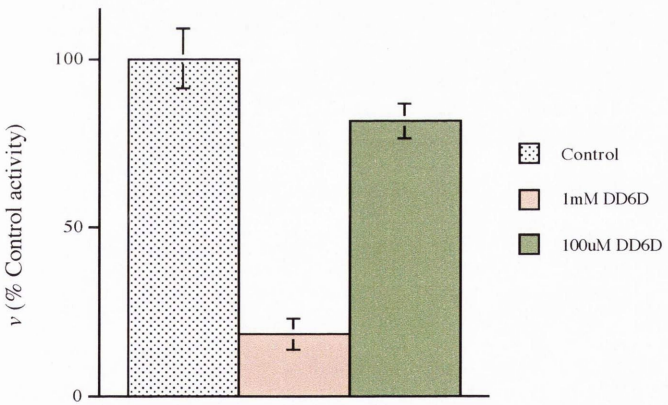
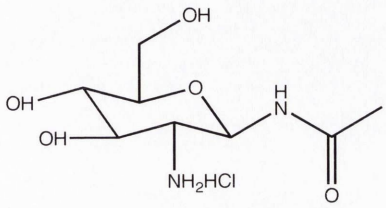

v (% Control activity)
100
50
0
Control
1mM DD6D
100uM DD6D

Fig. 3.7.18 The effect on the initial rate SSAO (partially purified) oxidative deamination of benzylamine by the amino sugar DD6E (see structure below) synthesized by Dr. Paul Murphy, Conway Institute, University College, Dublin.

The sugar (1 mM and 100 μM) was incubated for 5 min with 0.01mg/ml of protein at 37 ^{o}C. The reaction was initiated by the addition of 20 μM of ^{14}C-labelled benzylamine with a specific activity of 0.3 μCi/μmol. The reaction was terminated after 10 min by the addition of 100 μl of 2 M citric acid (Tipton *et al.*, 2000). Samples were the extracted in scintillant and counted on TriCarb 2100 scintillation spectrophotometer, as described in Section 2.4.4. Each value is the mean ± S.E.M. (n = 3)


OH
OH
OH
O
H
N
NH2HCl
O

DD6E-2(+HCl)

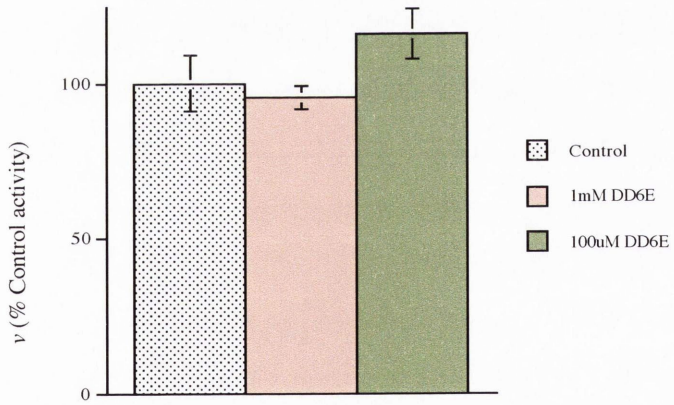
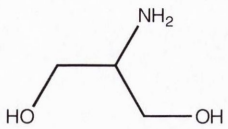

v (% Control activity)
100
50
0
Control
1mM DD6E
100uM DD6E

Fig. 3.7.19 Determination of the effect of serinol on the oxidative deamination of benzylamine by SSAO (partially purified).

Protein (0.1mg/ml) was preincubated with the stated concentrations of serinol for 5 min. The reaction was initiated by the addition of 5mM benzylamine. Initial rates were determined by following the production of a quinoneimine dye (molar absortivity = 4654 M^{-1} cm^{-1}) at 498nm (Holt *et al.*, 1997) on a SpectraMax 340PC plated reader as described in Section 2.4.8. Each value is the mean ± S.E.M. from five separate experiments.


NH2
HO
OH

serinol

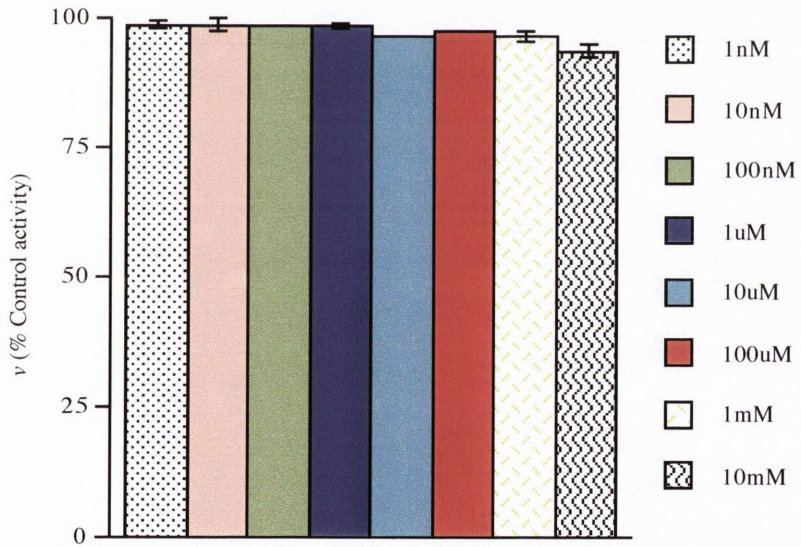
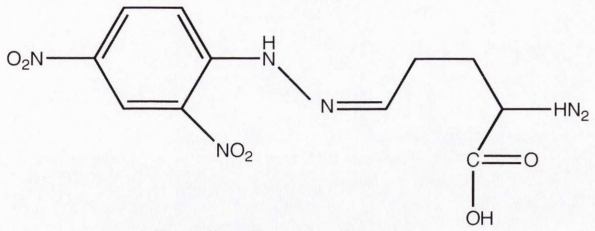
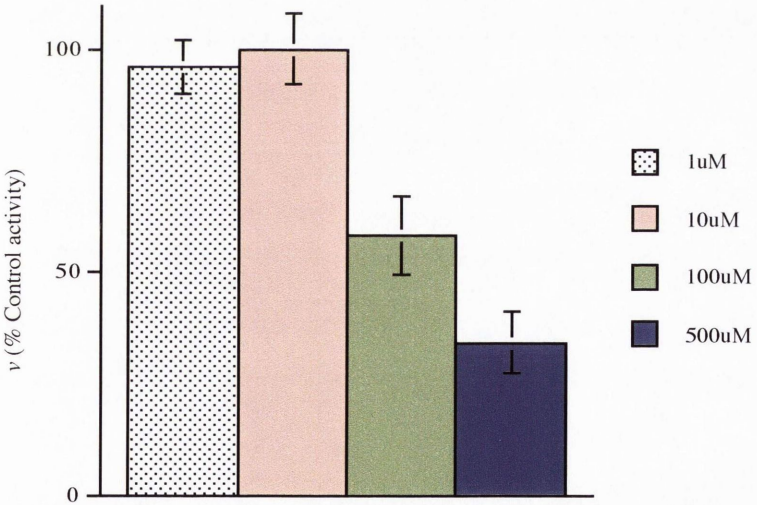

100
75
50
25
0
v (% Control activity)
1nM
10nM
100nM
1uM
10uM
100uM
1mM
10mM

Fig. 3.7.20 Determination of the effect of pyrroline-5-carboxylic acid on the oxidative deamination of benzylamine by SSAO (partially purified).

Protein (0.1 mg/ml) was preincubated with the stated concentrations of pyrroline-5-carboxylic acid for 5 min. The reaction was initiated by the addition of 5 mM benzylamine. Initial rates were determined by following the production of a quinoneimine dye (molar absortivity = 4654 M^{-1} cm^{-1}) at 498nm (Holt *et al.*, 1997) on a SpectraMax 340PC plated reader, as described in section 2.4.8. Each value is the mean ± S.E.M. from five separate experiments



Pyrroline-5-carboxylic acid



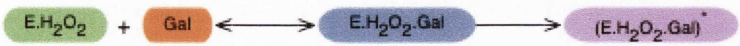
3.8 Discussion

Mannosamine, glucosamine and galactosamine were found to be effective time-dependent inhibitors of SSAO activity, whereas their *N*-acetyl derivatives and the unsubstituted hexoses failed to inhibit the enzyme. This and the different potencies of inhibition by the amino sugars, suggest that the inhibition shows specificity for the structure of the amino sugar.

The inhibition of SSAO by galactosamine was found to be time-dependent but fully reversible. However, the inhibition observed when the amino sugars were present in an assay mixture was not obtained when the enzyme was preincubated with galactosamine for extended times before the reaction was started by the addition of substrate. Since, galactosamine does not appear to be a substrate for SSAO, these results might suggest that one, or more of the products of the SSAO-catalysed amine oxidation might be necessary for the inhibitory process to occur. Two of the reaction products of benzylamine oxidation, benzaldehyde and ammonia were found to have no effect on the inhibition of SSAO by galactosamine. Preincubation of the enzyme with galactosamine plus H_2O_2 was, however, found to result in time-dependent decreases in the initial rates, when benzylamine was added to start the reaction. Furthermore this inhibition was prevented by the presence of catalase. A smaller degree of inhibition of SSAO was observed when it was incubated with some batches of galactosamine alone, but this was prevented when catalase was present. Thus inhibition might possibly result from H_2O_2 formation, which has been reported to be formed as the result of a non-enzymatic breakdown of the amino sugar itself (Hiraku et al, 1999). This process appeared to be supported, under the conditions used here, by the demonstration of time-dependent formation of H_2O_2 fluorimetrically when such a sample of galactosamine (5 mM) was incubated in the absence of SSAO. However, other samples of galactosamine, from the same supplier, which did inhibit SSAO when present in the complete assay mixture, showed no significant formation of H_2O_2, or time-dependent inhibition when preincubated with the enzyme in the absence of added H_2O_2. Thus, it was assumed that this process was catalysed by an impurity present in some, but not other, galactosamine samples.

These results suggest that the H_2O_2 formed as a product of the SSAO-catalysed oxidation reaction is necessary to enable the inhibition of the enzyme by galactosamine. In order to detect differences in the initial rates of substrate oxidation it was necessary to add substantially more exogenous H_2O_2 (1-5 mM) than would be produced by the oxidation of benzylamine alone (59.6 μM produced.h^{-1} under the standard assay conditions used in this work). This suggests that H_2O_2 formed at the active site of the enzyme, during the reaction, is situated in a position where it can interact with the amino-sugar in a productive manner and that considerably more exogenous H_2O_2 is necessary to achieve a similar local concentration at this site. This may result from a poor accessibility of the site at which H_2O_2 is formed (Li *et al.*, 1998, Wilice *et al.*, 1997), although the possibility that galactosamine may bind with greater affinity to an oxygen-containing enzyme intermediate in the reaction pathway cannot be excluded.

Addition of catalase after inhibition by galactosamine in the presence of benzylamine resulted in only a small regain in activity, which might suggest either that the H_2O_2 generated during this process is not readily accessible to catalase or that the inhibitory species is generated through reaction between H_2O_2 and the amino sugar. However, preincubation of H_2O_2 and galactosamine for 24 hours did not have any effect on the initial rates of the reaction when the reaction was started by the addition of SSAO and benzylamine. Therefore, it is unlikely that a non-enzymatic reaction between H_2O_2 and galactosamine would account for the inhibition process, but rather the slow formation of an (E. H_2O_2.Gal)* species, as outlined by the proposed reaction below, occurs:



The finding that the time-dependent inhibition exhibits saturation kinetics equations, as shown in equations (2) and (3), rather than (4) and (5), of Section 3.6.1, would be consistent with the amino-sugar forming a weak noncovalent complex with an intermediate enzyme species formed during the catalytic reaction and subsequently being transformed more slowly to a tighter associating, but still non-covalent complex.

The simplest models that could account for this process would involve interaction of the amino sugar with an enzyme-product complex or a modified form of the enzyme that is formed during the normal substrate oxidation cycle. Such uncompetitive mechanisms are shown in Fig. 3.8.1. The possibility that inhibition and substrate oxidation occur as parallel processes, where inhibitor and substrate compete for the free enzyme, as shown in 3.8.2, can be excluded because of the requirement for the enzyme-catalysed reaction to be proceeding if inhibition is to be observed.

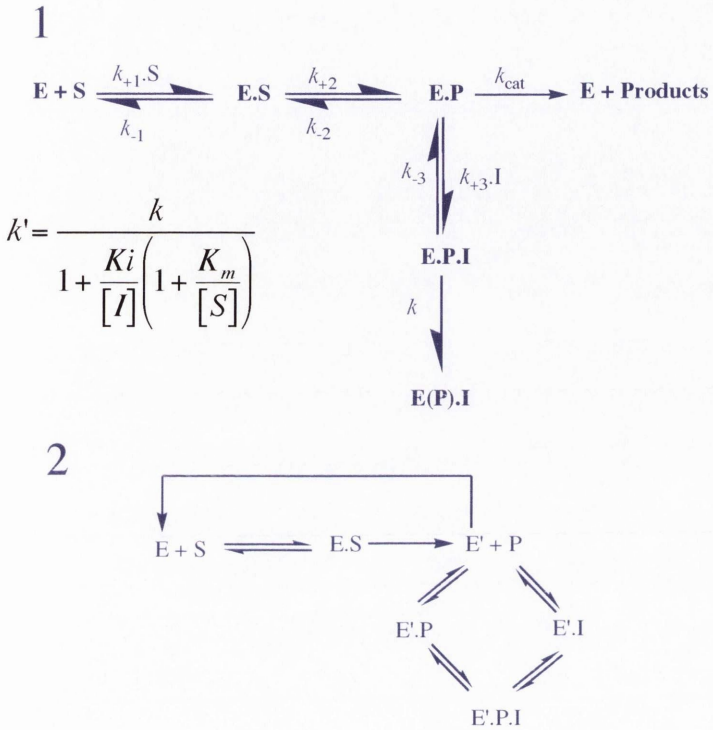
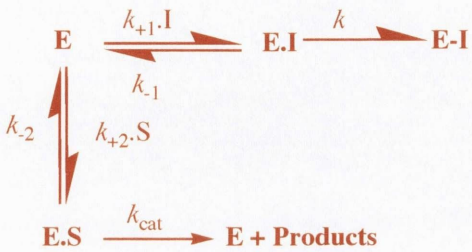


Fig. 3.8.1 Possible uncompetitive mechanisms for the inhibition of SSAO by galactosamine.

In mechanism (1) the amino sugar interacts with an enzyme-H_2O_2 complex, whereas in mechanism product (H_2O_2) is assumed to be capable of dissociating from and reassociating with the enzyme. Under steady-state, rather than rapid-equilibrium, conditions mechanism (2) would give to complex rate equations and departure from the simple hyperbolic relationship between K^{app} and galactosamine concentration that was observed. P = H_2O_2, S = substrate and I = galactosamine.

1



$$k' = \frac{k}{1 + \frac{K_i}{[I]}\left(1 + \frac{[S]}{K_m}\right)}$$

Fig. 3.8.2 A possible competitive mechanism for the inhibition of SSAO by galactosamine. P = H_2O_2, S = substrate and I = galactosamine.

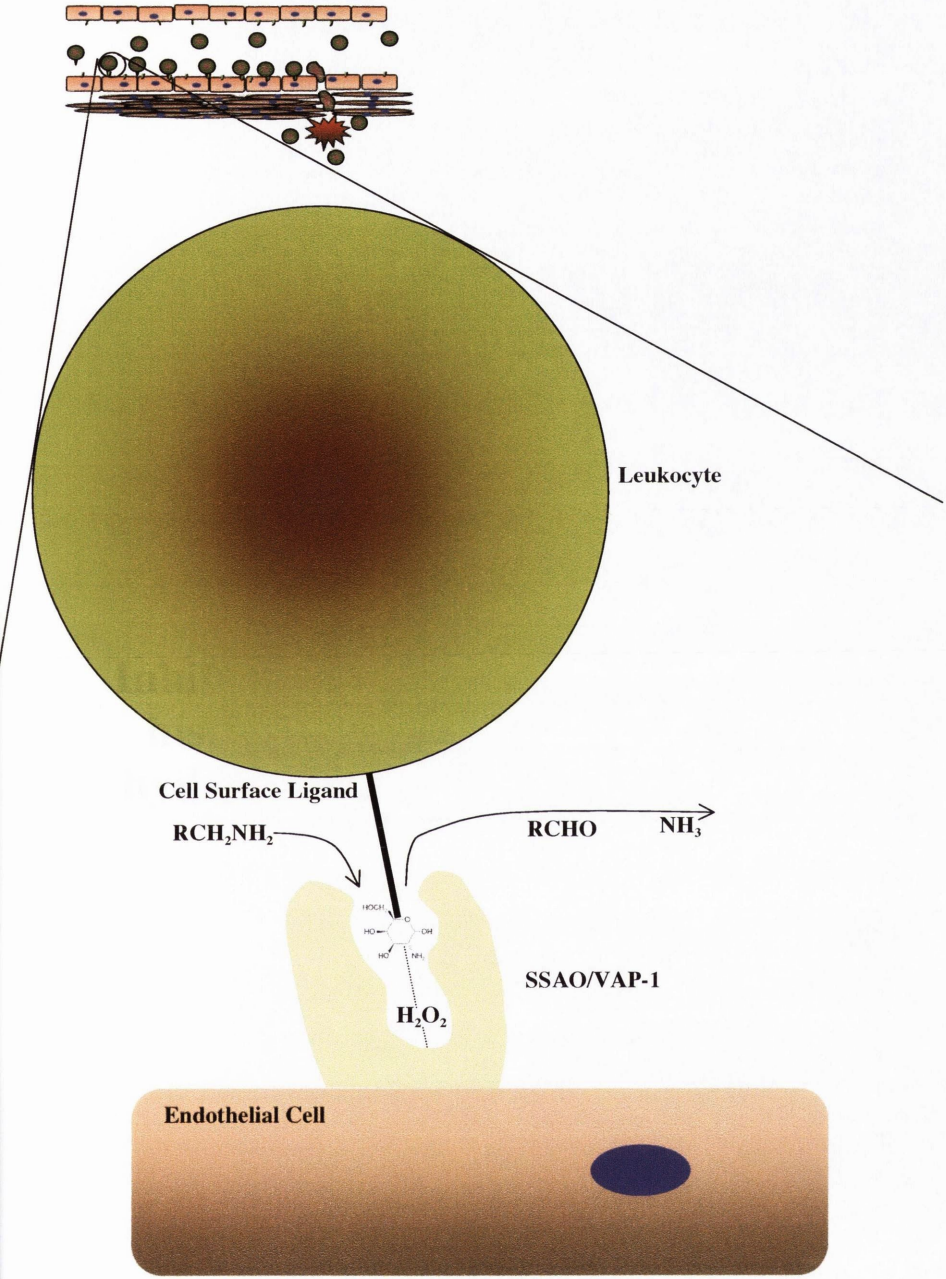
The time dependence and difference in potency displayed by amino sugar inhibition of SSAO suggest specificity towards the structure of sugar. Several compounds were tested as possible analogues. These were selected on the basis of some structural or partial structural similarity with the amino sugars. The effects of three non-physiological amino sugars, which had been synthesised by Dr. Paul Murphy, on SSAO activity were analysed. The three sugars are structurally different from the physiologically occurring compounds, with the closest in similarity being DD6B. Unlike the physiological sugars none of these synthetic analogue sugars showed any time-dependent inhibition during the oxidation of benzylamine by SSAO, however DD6B and, to a lesser extent, DD6D were concentration-dependent inhibitors of the enzymatic reaction.

Serinol shows partial similarity to the general amino sugar structure but is smaller than the sugars studied. SSAO was not inhibited by serinol even at concentrations up to 10 mM. However, pyrroline-5-carboxylic acid, a much larger molecule, inhibited the oxidative deaminiation of benzylamine by SSAO, although not in a time dependent manner, with an IC_{50} value of approximately 250 μM.

The failure of the free hexes and the *N*-acetyl-amino hexoses, as well as serinol and pyrroline-5-carboxylic acid, to act as time-dependent inhibitors suggests the presence of a free amine group to be important in this respect. However, this is clearly not sufficient, since none of the three 2-amino sugar analogues tested were time-dependent inhibitors. In order for a substrate molecule to reach the active site of the enzyme it is believed necessary for it to traverse an entrance cavity (Parsons *et al.* 1995). This may also be the case with the inhibitory amino sugars; allowing them to interact with the active site of the enzyme for the formation of the $E.H_2O_2.Gal$ species. A more bulky molecule, such as pyrroline-5-carboxylic acid, although capable of binding to the enzyme as a simple inhibitor, may not be able to penetrate the entrance cavity sufficiently for the active-site interactions that lead to time dependent inhibition to occur. Clearly size is not the only factor involved in the simple inhibitory interactions with SSAO, since of the three 2-amino sugar analogues tested, the noninhibitory DD6E is a smaller molecule than DD6D, which did inhibit. Now that the crystal structure of SSAO/VAP-1 has been determined, modelling studies may help to resolve the factors important for simple and time-dependent inhibition.

This work suggests that cell-surface amino-sugar derivatives may be involved in the interaction between VAP 1 (present on endothelial cells) with circulating lymphocytes. It has been suggested that H_2O_2 may mediate the process of vascular adhesion (Jalkanen and Salmi, 2001). The present results suggest that it is the hydrogen peroxide generated from amine oxidation by the action of SSAO itself that may potentiate its vascular-adhesion (VAP-1) function in a manner similar to that shown in Fig. 3.8.3.

Fig 3.8.3 Schematic representation of proposed mechanism for the interaction of galactosamine with SSAO/VAP-1, facilitating binding of leukocytes to endothelial cells mediated by the H_2O_2 produced during the enzymatic oxidative deamination of an amine.


Leukocyte
Cell Surface Ligand
RCH_2NH_2
RCHO
NH_3
SSAO/VAP-1
H_2O_2
Endothelial Cell

Chapter 4

Inhibition of amine oxidases by the histamine-1 receptor antagonist hydroxyzine and a possible role in multiple sclerosis

4.1 Enzyme inhibition

Enzyme activity can be modulated by several means, such as pH and temperature, but of primary interest in regards to this study are the role of inhibitors. Inhibitors affect the activity of an enzyme by combining with it in such a way that catalytic activity is decreased. The simplest forms of enzyme reversible inhibition are competitive, and mixed (also sometimes known as noncompetitive). Simple competitive inhibition occurs when a substance binds reversibly to the substrate-binding site an enzyme. Structurally the inhibitor is normally similar to the substrate, in order to allow it to bind to the active site, but different in that it is unreactive. An uncompetitive inhibitor binds only to the enzyme-substrate complex and not to free enzyme. In contrast to competitive inhibition, an uncompetitive inhibitor does not need to resemble the substrate. Mixed inhibition results when an inhibitor can bind to both the active site, in a manner similar to competitive inhibition, but can also bind the enzyme-substrate complex. Reversible inhibitors can disassociate from their complexes with the enzyme and removal of free (unbound) inhibitor, such as, by metabolism or dilution will result in a regain of the enzyme activity. In contrast, irreversible inhibitors remain fixed to the active site and the inhibition is unaffected by removal of any free (unbound) inhibitor. *In vivo*, enzyme activity in this case will only return as a result of the synthesis of new enzyme molecules.

4.2 Catalytic mechanisms of the amine oxidases

4.2.1 Monoamine oxidase

Monoamine oxidase oxidatively deaminates numerous amines both exogenous and biogenic amines such as neurotransmitters. The reaction converts the amine into to its corresponding imine, which is then nonenzymatically hydrolysed to an aldehyde. See Fig. 4.1 for an outline of the reaction catalysed by monoamine oxidase.

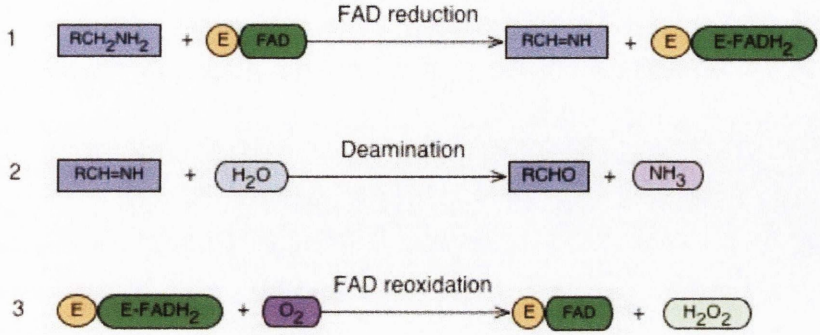
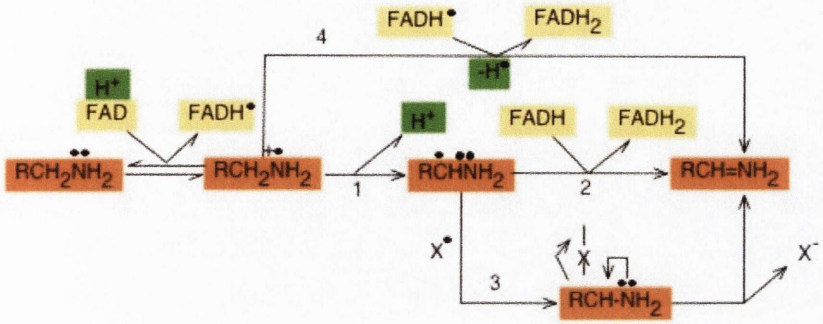


Fig. 4.1. Reaction catalysed by monoamine oxidase

Present day theories regarding the catalytic mechanism of MAO are divided between a single electron transfer mechanism (SET) (Rigby *et al.*, 2005), hydrogen atom transfer (HAT) and a proposed nucleophilic mechanism (Edmondson *et al.*, 2004). There has been much controversy about the validity of the SET pathway. In this the initial step proceeds via an single-electron transfer step from the lone pair of the substrate to the oxidized flavin which generates an aminyl radical cation and the flavin semiquinone FAD. Deprotonation of the α-carbon yields the α-amino radical which can choose one of two routes to follow: either a transfer of a second electron to the semiquinone, resulting in a reduced flavin radical ($FADH^-$), or formation of a species with an active site radical, producing a covalent adduct, which in turn, should decompose forming the iminium ion (see pathway 1 in Scheme 1; Silverman, 1995). Doubts existed as to the existence of this mechanism as none of the radical intermediates could be detected (Tan *et al.*, 1983). Furthermore, there was no convincing experimental evidence for the flavin-semiquione intermediate (Ramsay *et al.*, 1986). However, recent studies have presented spectroscopic evidence of a stable tyrosyl radical, which supports the SET mechanism (Rigby *et al.*, 2005).

The HAT mechanism is illustrated in Scheme 1, as pathway 4, and was put forward as a possibility when *trans*-(1-9-aminomethyl)-2-phenylcyclopropane was used to determine

the fate of the *trans*-(2-phenylcyclopropyl) carbinyl radical during a MAO-A catalysed reaction, since HAT would be an alternative if the radical intermediate is bypassed, resulting in the production of the corresponding cyclopropylimine. This imine was produced and detected (Zelechonok & Silverman 1992) and, coupled with some of the deficiencies in the proposed SET mechanism, a HAT mechanism was proposed as an alternative. This suggests that the aminyl radical cation is not an obligatory part of a MAO-catalysed oxidative deamination. A direct hydrogen abstraction from the substrate producing the α-radical is quickly followed by a rapid electron transfer from the aminyl substrate radical, forming the flavin hydroquinone and protonated imine product (Franot *et al.*, 1998). Arguments for the nucleophilic-based mechanism centre on chemical models, which show that primary and secondary amines can undergo oxidation via nucleophilic mechanisms. This mechanism proposes that the amine substrate forms an adduct with FAD and that subsequent cleavage results in the formation of $FADH_2$ and the iminium metabolite (Kim *et al.*, 1994). X-ray crystallographic work has suggested that the approach to the active site by a suitable substrate will result in a nucleophilic mechanism, because the amino-acid side chains necessary for the alternative mechanisms are not sufficiently close. The 3-D structure of MAO B shows two hydrophobic cavities, an entrance cavity and an active site cavity. Both cavities can appear as fused or as separate spaces, depending on the conformation of an Ile 199 side chain which can function as a gate between the cavities (Hubalek *et al.*, 2005). Once bound, the amino group of the substrate approaches a contorted FAD via an aromatic cage formed by two tyrosine residues perpendicular to the plane of the flavin ring. The description of this process concludes that, within the active site, there are no amino acids available to act as either acids or bases. This, coupled with other structural data, supports the proposed nucleophilic mechanism (Edmondson *et al.*, 2004).

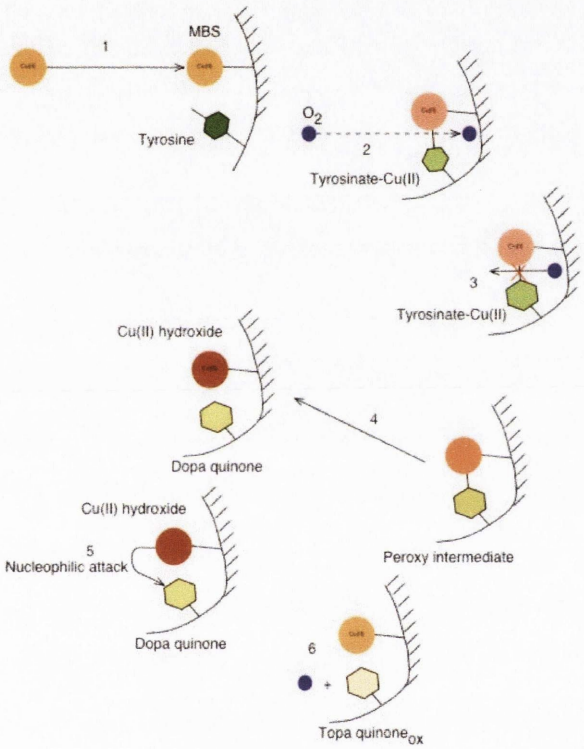


Scheme 1. Proposed mechanisms for a MAO catylsed reaction. See text for commentary (Silverman, 1995)

4.2.2 Semicarbazide-sensitive amine oxidase

Although both SSAO and MAO oxidatively deaminate amines and, in some cases, the same amines, via a similar two-step mechanism, with both oxidative and reductive half reactions, they are structurally different (as described in Section 1.3). The main difference regarding catalytic activity relates to the differing co-factors involved. The co-factor involved in the catalytic action of MAO is FAD that is derived form the vitamin B_2 (see Section 1.2), whereas the co-factor in SSAO is TPQ. Several possible mechanisms for the production of the TPQ co-factor have been presented (Janes *et al.*, 1990). Although it is still not fully elucidated, the most accepted mechanism of TPQ biogenesis is as summarised in Scheme 2. The copper within the SSAO molecule plays an essential role in the production of the TPQ co-factor. The steps in the process are (1) the entrance of Cu(II) to the metal-binding site (MBS) of the enzyme, which occurs before the arrival of O_2. This allows the Cu (II) to be correctly positioned and prevents the precursor tyrosine becoming a ligand for the Cu(II). (2) O_2 is introduced which causes a shift in the position of the precursor tyrosine allowing it to become a ligand for the Cu(II). This results in the formation of a tyrosinate-Cu(II) intermediate, which reacts with O_2. (3) This is believed to form an unstable peroxy intermediate. (4) This, then, breaks down to form

dopa quinone and Cu(II)-hydroxide. (5) a final nucleophilic attack on the dopa quinone by hydroxide generates the reduced TPQ. (6) This can be oxidized by O_2 to produce the oxidized form of TPQ, which is its resting state within the SSAO molecule (Dove *et al.*, 2000).



Scheme 2. Stages in the biosynthesis of the TPQ co-factor in copper containing amine oxidases. Based on the mechanism described by Dove *et al* 2000.

Once formed the TPQ in its oxidized form is prepared for use in the catalytic reaction of SSAO. Unlike the mechanism behind MAO catalysis, which is still a matter of some debate, the mechanism of SSAO appears to be more straightforward. Illustrated in Fig. 4.2 are the general stages of catalysis of SSAO.

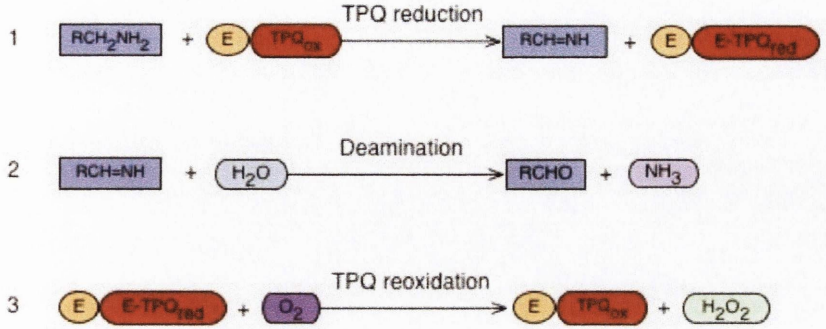
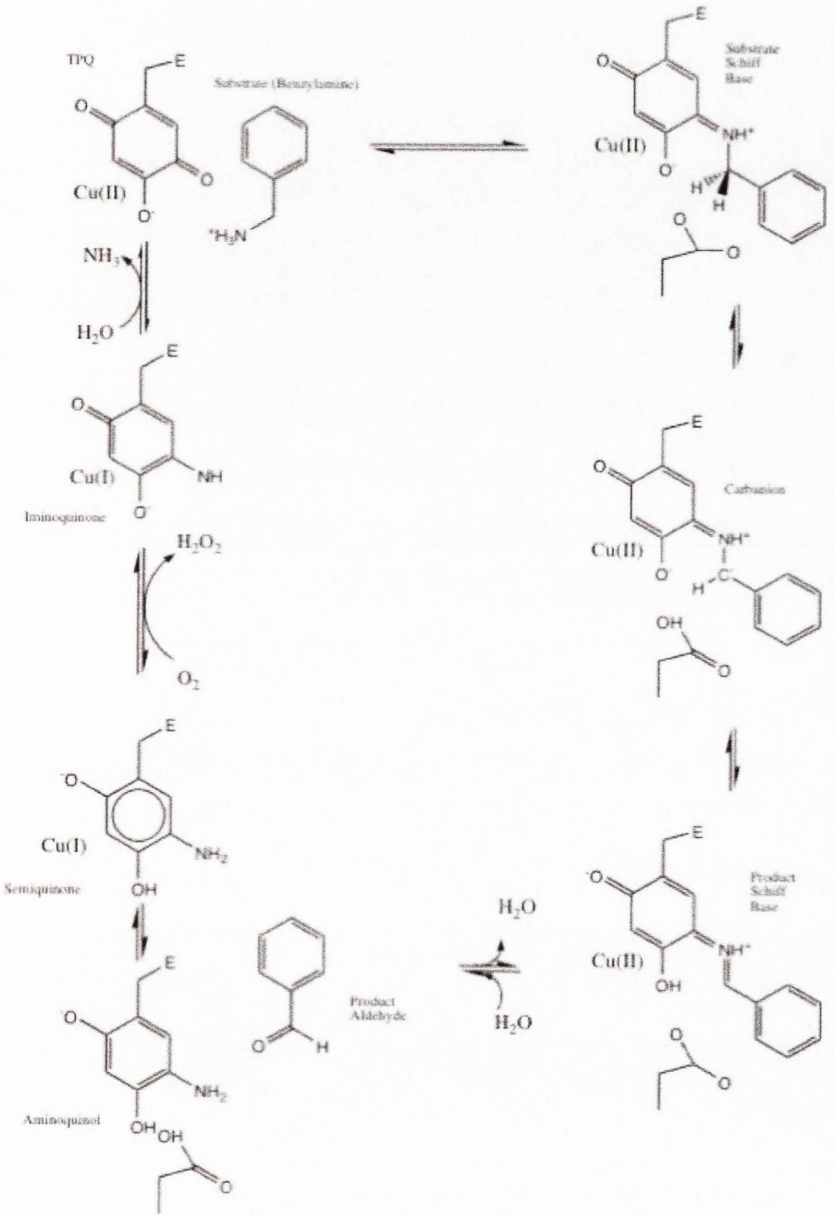


Fig. 4.2. Reaction catalysed by semicarbazide sensitive amine oxidase

The stages of SSAO oxidation are graphically represented in Scheme 3, where the mechanism is illustrated in the presence of the substrate benzylamine. The initial step in the oxidation of the substrate is the formation of a substrate Schiff base as a result of a nucleophilic attack of the substrate by C5 of the TPQ within the enzymes active site. After the formation of the substrate Schiff base, a conserved aspartic acid residue (D319) (Mure *et al.*, 2002), acting as an active-site base, abstracts a proton forming a carbanionic species, which can quickly rearrange to form a corresponding product Schiff base. At this stage in the catalytic cycle the TPQ is now reduced to the aminoquinol state and the first product of the reaction, the aldehyde, is produced through a condensation reaction.

Scheme 3. Reaction pathway of the oxidative deamination of benzylamine by semicarbazide sensitive amine oxidase. Modified from Brazeau *et al.*, 2004.


TPQ
Substrate (Benzylamine)
Cu(II)
NH_3
H_2O
Substrate Schiff Base
Iminoquinone
Cu(I)
H_2O_2
O_2
Carbanion
Semiquinone
NH_2
OH
Product Aldehyde
H_2O
Aminoquinol
Product Schiff Base

The reaction up until now is considered as the reductive half of the reaction. It leads directly into the oxidative half reaction. Molecular oxygen binds to the enzyme and accepts two protons from the aminoquinol intermediate, producing hydrogen peroxide and an iminoquinone intermediate. Hydrolysis of the iminoquinone intermediate yields ammonia and results in the re-oxidation of the TPQ, allowing it to participate in another catalytic cycle (Brazeau *et al.*, 2004).

4.3 Amine oxidase inhibition

Much interest has focused on the effects caused by the inhibition of both SSAO and MAO. Already discussed are the implications of both SSAO and MAO inhibition (see Chapter 1), so within this section a more detailed description of some of the mechanisms of inhibition encountered by the amine oxidases will be described.

4.3.1 Reversible inhibitors of MAO

Inhibition of amine oxidases can be either reversible or irreversible. As described above, reversible inhibitors may be competitive, uncompetitive or mixed in type. Competitive inhibitors are generally believed to bind to the same site as the substrate, but the physical impairment of amine oxidase activity may also be due to the route a substrate has to travel to reach the active site. In both SSAO and MAO crystal structures a channel (Lunelli *et al.*, 2005) or deep cavity (Binda *et al.*, 2004) have been identified through which any substrate must transverse before it will be oxidized in the active site. Therefore an inhibitor may reduce the enzyme activity simply by blocking these entrances and physically preventing substrate access.

Reversible inhibitors of MAO A, such as the slow-binding inhibitor moclobemide (Cesura *et al.*, 1990), have found clinical use in the treatment of depression because they minimise the adverse interactions with dietary amines that have been a problem with irreversible inhibitors of this enzyme (see Da Prada *et al.*, 1990). 2-amino-ethyl carboxamide derivatives, such as the selective MAO-A and -B inhibitors Ro41-1049 and Ro16-6491, respectively, have a more complicated mechanism. They are oxidised by MAO to give more potent, but reversible, inhibition (Cesura *et al.*, 1990).

4.4.2 Irreversible inhibitors of MAO

The widely recognized and commonly used irreversible inhibitors of MAO are clorgyline (MAO A), deprenyl (MAO B) and pargyline (weaker preference for MAO B), These are mechanism-based or suicide inhibitors of the enzymes. Suicide inhibitors act as substrates but a reaction intermediate formed through the reaction with the enzyme is the inhibitory species involved. For the MAO inhibitors mentioned the mechanism is believed to be the formation of an adduct with N-5 of the isoalloxazine the cysteinyl-flavin in the active site of the enzyme (Maycock *et al.*, 1976)

Further studies on MAO inhibition by cyclopropylamine and α-silylamine propose a polar mechanistic model (Kim *et al.*, 1995) similar to that of the hydrazine mechanism. In the case of phenylhydrazine, the enzyme oxidises it to phenyldiimide, which then reacts to form a stable adduct with the reduced flavin. Other hydrazine derivatives behave similarly, but the process with aralkhydrazine is believed to involve diazene intermediates (Patek *et al* 1974).

Another proposed mechanism of inhibition observed in MAO oxidation has been elucidated by studies of the metabolism 3-aryl-Δ^3-pyrrolines, which are structural analogs of MPTP a by MAO. It was suggested that proceeds by two one-electron oxidation steps to form the corresponding pyrrolium cation. This ion could then lose a proton, generating the corresponding pyrrole or be trapped by water or by a nucleophilic species on the enzyme, which would result in the inactivation of MAO (Williams & Lawson 1998).

4.4.3 Irreversible inhibitors of SSAO

A family of 4-(aryloxy)-2-butynamines are suicide inhibitors of SSAO, where oxidation of the inhibitor forms a highly-reactive aldehyde, which can then form a covalent attachment to the reduced TPQ, preventing its reoxidation (O'Connell *et al.*, 2004). 2-Bromoethlyamine (2-BEA) is a suicide inhibitor of SSAO (Tabata *et al.*, 2003), which probably acts similarly. Sulphide and azide, which are time-dependent inhibitors act by binding to the the copper in SSAO (Dooley & Cote 1984). The classic inhibitors of

SSAO, the carbonyl compounds such as semicarbazide and cyanide inhibit the enzyme by competing with O_2 for the reduced TPQ after complexing with the copper preventing the reoxidation of the TPQ (Shepard *et al.*, 2004). An interesting mechanism of inhibition of SSAO occurs with 3-pyrrolines, which are substrates for the FAD containing MAOs. These compounds inhibit SSAO by pyrrolylation of the TPQ co-factor, in a process that resembles pyridoxal-dependent transamination (Lee *et al.*, 2001).

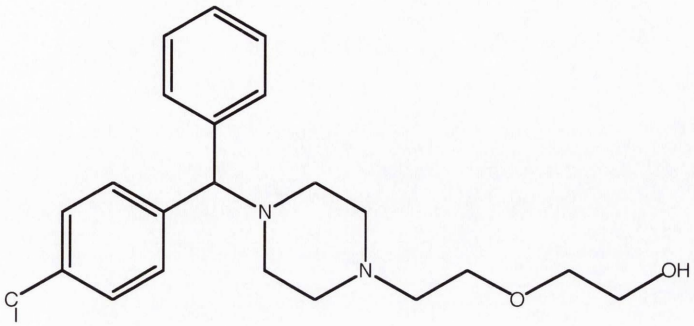
4.5 Hydroxyzine

The heterocyclic piperazine derivative hydroxyzine (2-[2-[4-[(4-chlorophenyl) phenyl-methyl]-1-piperazinyl] ethoxy] ethanol) (Fig. 4.3) is a histamine-1 receptor antagonist, which is in use as an antihistamine, usually in its acid form (Cetirizine) (Presa, 1999). It has a variety of therapeutic applications. When acting as a H_1 antagonist hydroxyzine (in its carboxylated form) and its enantiomers have shown extremely high selectivity towards the H-1 receptor resulting in a reduction in sedation and brain penetration (Gillard *et al.*, 2001), but cetirizine and its enantiomer are slowly reversible (Christophe *et al.*, 2003). Hydroxyzine is also used as a tranquilliser for the symptomatic management of conditions, such as generalised anxiety disorder (GAD) and the tension associated with psychoneuroses (Lader *et al.*, 1998), as well as in premedication associated with GAD. It is also used in the management of pruritis associated with allergic conditions, such as chronic urticaria and in histamine-mediated pruritus.

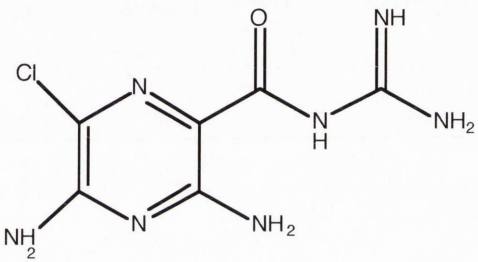
Hydroxyzine has also been shown to inhibit neurogenic mast-cell activation by a mechanism that is unrelated to its H-1 receptor antagonistic properties (Minogiannis *et al.*,, 1998). Recent studies have shown that hydroxyzine may also suppress inflammatory responses as it possesses an ability to inhibit both macrophage inhibitory factor (MIF) and IL-8 production (Shimizu *et al.*, 2004). This was further confirmed by the demonstration of down regulation of inflammatory responses in keratinocytes by hydroxyzine and its enantiomers (Giustizieri *et al.*, 2004). It has been shown to inhibit experimental allergic encephalomyelitis (EAE) and the associated brain mast cell activation (Dimitriadou *et al.*, 2000). Preliminary results have also shown it to be effective in the prevention of relapsing/remitting multiple sclerosis (Dimitriadou *et al.*,

2000). However, some of these 'first-generation' antihistamines, including hydroxyzine, can act as K^+ channel blockers and have also been implicated in adverse effects within the cardiovascular system and the CNS (Taglialatela *et al.*, 2000),

Histamine appears to play an important role in cell adhesion (see Andriopoulou *et al.*, 1999; Winter *et al.*, 1999). The link between histamine, which is a substrate for some semicarbazide-sensitive amine oxidases (Raimondi *et al.*, 1997), and adhesion, together with the role for SSAO as an adhesion protein, suggests the possibility that hydroxyzine, and some other histamine receptor antagonists, might interact with SSAO. If it were to be an inhibitor this might affect the various SSAO/VAP-1 functions.



A



B

Fig. 4.3. Chemical structure of (A) Hydroxyzine (2-[2-[4-[(4-chlorophenyl) phenyl-methyl]-1-piperazinyl] ethoxy] ethanol). (B) Amiloride (shown to indicate the lack of similarity).

4.6 Multiple Sclerosis

First described in 1868 by Jean Martin Charcot, multiple sclerosis (MS) was observed as an accumulation of inflammatory cells within the brain and the spinal cord along with plaque formation (Charcot 1868). The, seemingly, inflammatory nature of the condition was further developed during the 1940s by Elvin Kabat, who observed an accumulation of an oligoclonal immunoglobulin in the cerebrospinal fluid of patients (Kabat *et al.*, 1948). The origins of the disease were originally uncertain. However, the development of an animal model for the disease, known as experimental autoimmune encephalomyelitis (EAE), resulted in the, now generally accepted, hypothesis that MS is a secondary response to self-antigens in genetically susceptible hosts (Rivers *et al.*, 1933). The predominant pathological characteristic of the disease is the demyelination of the central nervous system. This is believed to be initiated by an unknown agent/s which stimulates an immune response. The sequence of events generally occurs as (1) initial disruption of the blood brain barrier (2) followed by an influx of T-cells (3) upon recognition of an antigen by the T-cells (4) a cytokine cascade (5) further stimulates the immune response (6) resulting in damage to the myelin . This is summarised in Fig. 4.4. It has been suggested that a viral agent may be the initial triggering factor. This would then result in the activation of myelin reactive T-cells in the peripheral immune system, as shown in experimental animal models (Tejada-Simon *et al.*, 2000).

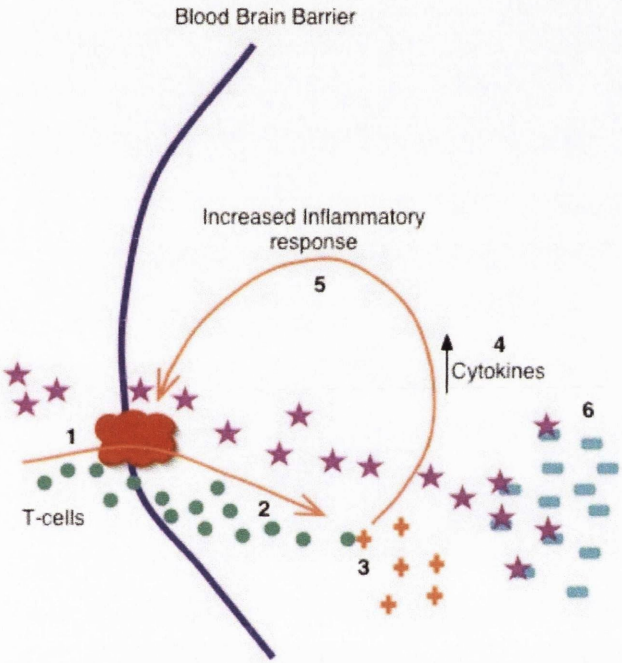


Fig. 4.4. Graphical outline of the onset of multiple sclerosis. The sequence of events occurs as (1) initial disruption of the blood brain barrier (2) followed by an influx of T-cells. (3) Upon recognition of an antigen by the T-cells (4) a cytokine cascade (5) further stimulates the immune response (6) resulting in damage to the myelin.

4.7 Aims & objectives

Because of the apparent multiplicity of functions of SSAO, the behaviour of drugs, such as amiloride (Fig. 4.3) and some other imidazoline and guanidine compounds, that bind to and inhibit it might be complicated. This study was undertaken to investigate whether hydroxyzine, a compound with a different spectrum of actions from amiloride, was also an inhibitor of SSAO and/or MAO.

The role of SSAO as a adhesion protein under inflammatory conditions and the report that hydroxyzine reduces EAE in mice, suggested a possible link. Therefore, this work set out to determine a link between the adhesion ability of SSAO and a role in the progression of EAE/MS.

4.8 Materials and Methods

All enzyme assays were performed at 37^{o}C. SSAO activity towards benzylamine was assayed as described in Section 2.4.1.

The oxidation of 5-HT and PEA, substrates for rat liver mitochondrial MAO A and MAO B, respectively, were determined as described in Section 2.4.5.

Dilution experiments were performed in order to assess inhibitor reversibility against SSAO as described in Section 2.4.6.

Determination of the reversibility of hydroxyzine inhibition of MAO was studied as described in Section 2.4.7.

Dr. Brian Keogh (TCD) kindly prepared the histological samples in facilities made available in School of Veterinary Science, University College Dublin.

4.8 Results

4.8.1 Preliminary determination of the effect of the histamine-1 receptor antagonist, hydroxyzine, on the oxidative deamination of benzylamine by bovine plasma SSAO.

The H-1 receptor antagonist hydroxyzine caused a reduction in the activity of bovine plasma SSAO. The inhibition was observed by measuring the progress of the oxidative deamination of benzylamine to benzaldehyde by the enzyme at 250nM by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). The observed inhibition was concentration dependent in manner see Fig. 4.8.1.

4.8.2 Determination of the kinetic nature of the inhibition of bovine plasma SSAO by the histamine-1 receptor antagonist, hydroxyzine.

The inhibition of bovine plasma SSAO (from Sigma) by hydroxyzine was found to be time-independent with IC_{50} values of 17 ± 0.83 and 20 ± 0.85 μM after incubation for 0 min and 60 min, respectively (see Fig. 4.8.2). Determination of the K_i value for the oxidation of benzylamine by bovine plasma SSAO in the presence of hydroxyzine was completed by the measurement of the initial rates of oxidation at a series of substrate and inhibitor concentrations. Results are presented a Michaelis-Menten plot (Fig. 4.8.3) and as a double-reciprocal plot, for illustrative purposes only (Fig. 4.8.4). Inhibition was competitive and the K_i value for the inhibition of SSAO by the histamine-1 receptor antagonist, hydroxyzine was 3.8 ± 0.39 μM (see Fig. 4.8.5).

4.8.3 Effect of hydrogen peroxide on the inhibition of bovine plasma SSAO by the histamine-1 receptor antagonist, hydroxyzine.

One of the end products of the oxidative deamination of benzylamine by bovine plasma SSAO, hydrogen peroxide, was removed through the action of catalase to determine if its presence was required for the inhibition. Hydrogen peroxide removal had no effect on

the manner or potency of inhibition by hydroxyzine on bovine plasma SSAO (see Fig. 4.8.6).

4.8.4 Determination of the kinetic nature of the inhibition of partially purified bovine plasma SSAO by the histamine-1 receptor antagonist, hydroxyzine.

The inhibition of partially purified bovine plasma SSAO by hydroxyzine was found to be similar to that of the preparation from Sigma. The IC_{50} values were 23 ± 0.5 and 20 ± 0.42 μM after incubation for 0 min and 60 min, respectively (see Fig. 4.8.7). Determination of the K_i value for inhibition of the oxidation of benzylamine by this preparation of bovine plasma SSAO was as described in the previous section. Results are presented a Michaelis-Menten plot (Fig. 4.8.8) and as a double-reciprocal plot for illustrative purposes only (Fig. 4.8.9) The K_i value for the competitive inhibition of SSAO by the histamine-1 receptor antagonist, hydroxyzine was 1.47 ± 0.79 μM (see Fig. 4.8.10).

4.8.5 Amine oxidase activity in isolated bovine lung microsomes.

Bovine lung microsomes were prepared as a source of membrane-bound SSAO (Lizcano *et al.*, 1998) (see Section 2.6). Total amine oxidase activity was measure with and without clorgyline and deprenyl, to determine any contribution from MAO activity. A decrease in activity was observed in the presence of the MAO inhibitors, as benzylamine was the substrate in use this reduction in activity would be due to the inhibition of MAO B and, therefore, this necessitated the addition of the inhibitors (1 μM) into later studies using microsomes as a source of membrane bound SSAO.

4.8.6 Determination of the inhibition of isolated bovine lung microsomal SSAO by the histamine-1 receptor antagonist, hydroxyzine.

The inhibition of microsomal membrane bound SSAO by hydroxyzine was found to be time-independent with IC_{50} values of 1.0 ± 0.04 and 0.870 ± 0.03 mM after incubation

for 0 min and 60 min respectively (see Fig. 4.8.12). Microsomes were incubated in the presence of endoglycosidase-H for 20 hours and the effect on hydroxyzine inhibition of membrane bound SSAO were observed. An IC_{50} value of 2.9 ± .079 mM was obtained (see Fig 4.8.13).

4.8.7 The effect of endoglycosidase treatment on activity and hydroxyzine mediated inhibition of partially purified bovine plasma SSAO

Partially purified bovine plasma SSAO (1 mg/ml) was incubated with 1 unit of endoglycosidase-H in the presence and absence of hydroxyzine (20 μM). No significant differences in either enzyme activity when compared to the control or where hydroxyzine was included on the oxidative deamination of benzylamine (Fig. 4.8.14). After preincubation no change in the molecular mass of the partially purified bovine plasma SSAO was observed (Fig. 4.8.15).

4.8.8 Determination of the inhibition of rat liver MAO A by the histamine-1 receptor antagonist, hydroxyzine.

The inhibition of the oxidative deamination of 5-HT by rat liver miochondria was found to be time-independent with IC_{50} values of 631 ± 13 and 676 ± 16 μM after incubation for 0 min and 60 min respectively (see Fig. 4.8.16). The high IC_{50} value made determination of the K_i impracticable.

4.8.9 Determination of the nature of the inhibition of rat liver MAO B by the histamine-1 receptor antagonist, hydroxyzine.

The inhibition of the oxidative deamination of 2- phenylethylamine (PEA) by rat liver mitochondria with IC_{50} values of 40 ± 1.5 and 38 ± 0.9 μM after incubation for 0 min and 60 min respectively (Fig. 4.8.17). Determination of the K_i value for the oxidation of 2-phenylethylamine by rat liver mitochondrial MAO B in the presence of hydroxyzine was performed by measurement of the initial rates of oxidation over a range of substrate

and inhibitor concentrations. Results are presented as a double-reciprocal plot, for illustrative purposes in Fig. 4.8.18. Inhibition was competitive and the K_i value for the inhibition of MAO B by the histamine-1 receptor antagonist, hydroxyzine was 19 ± 1.3 μM (see Fig. 4.8.19).

4.8.10 Determination of the reversibility of the inhibition on amine oxidases by the histamine-1 receptor antagonist, hydroxyzine.

The inhibition of the amine oxidase preparations, used in this study, by hydroxyzine was found to be fully reversible with recovery rates after removal of the inhibitor. Inhibitor was removed by dilution for SSAO and by sedimentation for MAO. The enzymatic activity of SSAO was 80 ± 6% after hydroxyzine removal. MAO A activity was found to be 97.5 ± 1% after hydoxyzine removal and MAO B activity was restored to 65 ± 3.6% of the control activity (see Fig. 4.8.20).

4.8.11 Effect of the end products of MAO amine oxidation on the inhibition of bovine plasma SSAO by the histamine-1 receptor antagonist, hydroxyzine.

Rat liver mitochondria was incubated in the presence of the end products of MAO amine oxidation with and without hydroxyzine (1mM). Hydrogen peroxide, ammonia and aldehyde had no effect on the inhibition by hydroxyzine of MAO-A catalysed oxidative deamination of 5-HT (Fig. 4.8.21) or MAO-B catalysed oxidative deamination of 2-PEA (Fig. 4.8.22)

4.8.12 Inhibition of mouse brain SSAO in encephalomyelitis -induced C57 black mice by 2-bromoethlyamine.

Treatment of experimental allergic encephalomyelitis (EAE) induced C57 female black mice aged from 6-8 weeks, with the SSAO suicide inhibitor 2-bromoethylamine showed a significant decrease of 39% ($P = 0.0012$) in the SSAO activity of whole mouse brain homogenates towards benzylamine, when compared to the control, see Fig. 4.8.23.

4.8.13 Effect of 2-bromoethlyamine on the onset of EAE-induced in C57 black mice.

Comparison of the histological staining of sample sections of C57 black mouse brains, 30 days after induction with EAE, with and without treatment with 2-bromoethylamine was made (Fig. 4.8.24). Half brains were remove from mice and processed through standard histological techniques. Sections were mounted on slides and stained with hematoxylin and eosin. In the samples (n = 5) not treated with 2-BEA, the CNS white matter had many "holes" in it. These are vacuoles in the cell cytoplasm and are the hallmark of demyelination (Brok *et al.*, 2001). The large cellular infiltrate consists of lymphocytes (T and B), macrophages and neutrophils (Sobel *et al.*, 1984). This was previously reported as typical of inflammatory demyelination. However in the samples (n = 4) that had been treated with 2-BEA the white matter appeared normal and the border between the grey and white matter was clearly defined. There was no apparent demyelination of white matter neurons.

4.8.14 Effect of methlyhydrazine on SSAO activity in plasma, lung and brain tissue from EAE-induced C57 black mice.

An alternative inhibitor of SSAO, methylhydrazine, was administered to EAE-induced C57 female black mice aged from 6-8 weeks. The animals were sacrificed by cervical dislocation. As a result of apparent toxic effects of methlyhydrazine. Histology showed no differences between treated and untreated mice. However tissues taken from methylhydrazine treated mice showed a significant drop in SSAO activity in lung and brain and also in plasma. A mean drop of between 20 and 28% in SSAO activity towards benzylamine was observed in both plasma and brain, however no concentration dependent effect was detected (see Figs. 4.8.25 and 4.8.27). Lung tissue removed from the methylhydrazine treated mice did however show a concentration dependent inhibition that reached 58% in the mice receiving 0.25 mg/kg methylhydrazine (see Fig 4.8.26).

Enzyme	IC_{50} (0 min)	IC_{50} (60 min)	K_i
bp SSAO*	17 ± 0.83μM	20 ± 0.85μM	1.8 ± 0.39μM
pp SSAO*	23 ± 0.5μM	20 ± 0.42μM	1.47 ± 0.79μM
mb SSAO*	1.05 ± 0.043mM	0.870 ± 0.028mM	-
MAO A	631 ± 13μM	676 ± 16μM	-
MAO B	40 ± 1.5	38 ± 0.9μM	19 ± 1.3μM

Table 4.8.15. Table of kinetic parameters for the inhibition of amine oxidases by the histamine-1 receptor antagonist, hydroxyzine. *bp = bovine plasma (Sigma); pp= partially purified bovine plasma; and mb = membrane bound from beef lung

Inhibitor	Dose	Sample type	SSAO activity	Inflammation
2-bromethylamine	10mg/kg	Brain	↓ 39%	Reduced
Methylhydrazine	0.065mg/kg	Brain	↓ 27%	No difference
	0.125mg/kg		↓ 26%	No difference
	0.25mg/kg		↓ 28%	No difference
Methylhydrazine	0.065mg/kg	Lung	↓ 20%	-
	0.125mg/kg		↓ 44%	-
	0.25mg/kg		↓ 58%	-
Methylhydrazine	0.065mg/kg	Plasma	↓ 20%	-
	0.125mg/kg		↓ 20%	-
	0.25mg/kg		↓ 19%	-

Table 4.8.16. Summary of effects exhibited by SSAO inhibitors on encephalomyelitis -induced C57 black mice.

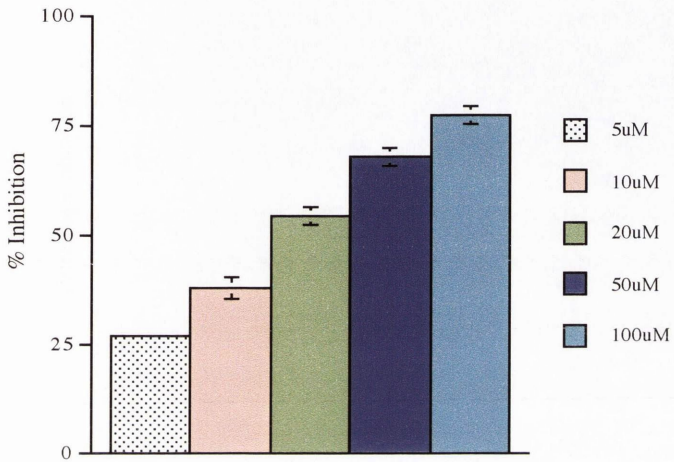


Fig. 4.8.1. The effect of the histamine-1 receptor antagonist hydroxyzine on the oxidative deamination of benzylamine by bovine plasma SSAO.

The enzyme (from Sigma; 0.0012 mg/ml protein) was preincubated with hydroxyzine for 5 min at 37°C before the reaction was started by the addition of benzylamine, to a final concentration of 5 mM. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954) and the effect on the initial rate was determined. Each sample is the mean value ± S.E.M. from triplicate determinations.

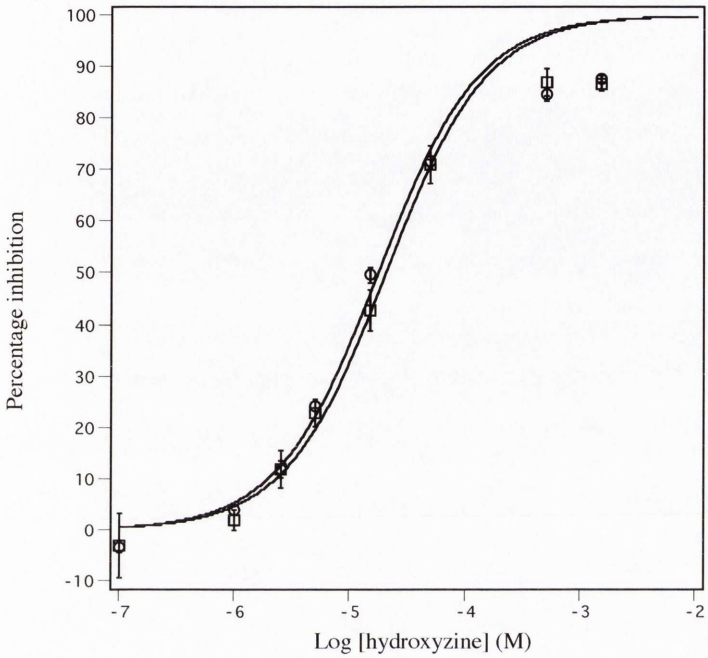


Fig. 4.8.2. The effect of incubation with hydroxyzine on the oxidative deamination of benzylamine by bovine plasma SSAO.

The enzyme (from Sigma; 0.0012 mg/ml protein) was preincubated with hydroxyzine for 5 min at 37°C before the reaction was started by the addition of benzylamine, to a final concentration of 5 mM. O = 0 min, □ = 60 min. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954) and the effect on the initial rate was determined. Each sample is the mean value ± S.E.M. from triplicate determinations.

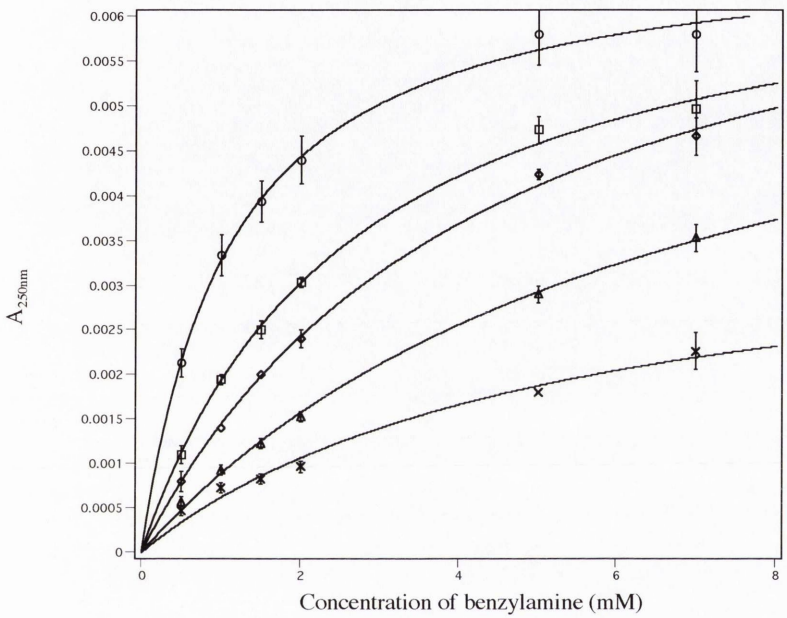


Fig. 4.8.3. The kinetics of the inhibition of bovine plasma SSAO by hydroxyzine.

The concentration of the enzyme (from Sigma) was 0.012 mg/ml protein. Initial rates of reaction were determined at the indicated concentrations of benzylamine and the following fixed concentrations of hydroxyzine 0 (O), 2.5 (□), 7.5 (◇) , 10 (Δ) and 15 (X) μM. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). Each point is the mean value ± S.E.M. from triplicate determinations.

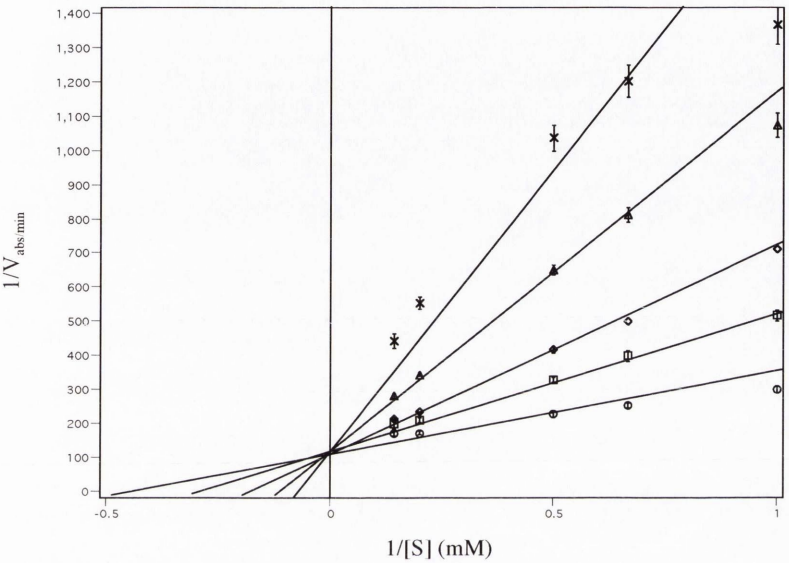


Fig. 4.8.4. Representation of the kinetics of the inhibition of bovine plasma SSAO by hydroxyzine as double-reciprocal plots.

The data from Fig. 4.8.3. are shown as a double-reciprocal plots for illustrative purposes. The concentrations of hydroxyzine were 0 (O), 2.5 (□), 7.5◇), 10 (Δ) and 15 (X) μM. Each point is the mean value ± S.E.M. from triplicate determinations. Data are shown as a double-reciprocal plot for illustrative purposes.

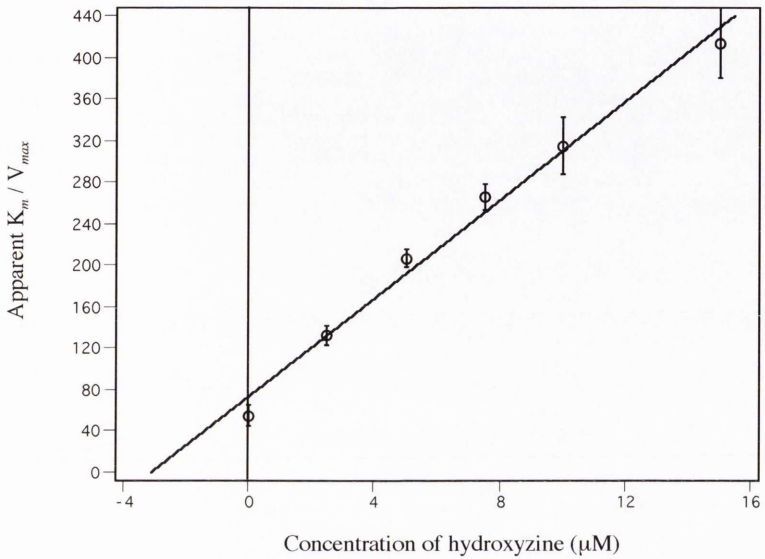


Fig. 4.8.5. Plot of the apparent values of K_m / V_{max} values determined from Fig. 4.8.3. as a function of the hydroxyzine concentration

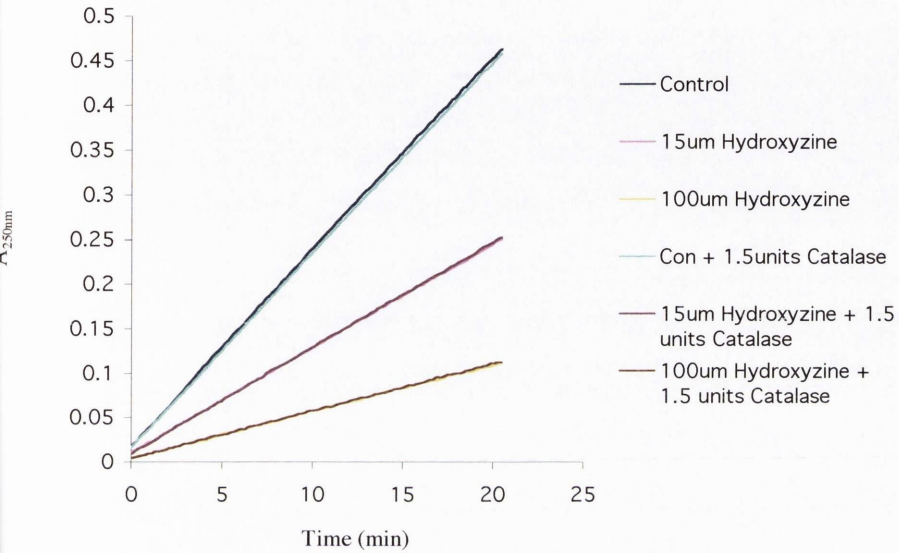


Fig. 4.8.6. The effects of hydroxyzine in the presence and absence of catalase on the reaction progress curves for the oxidative deamination of benzylamine by bovine plasma SSAO.

The concentration of the enzyme (from Sigma) was 0.012 mg/ml protein and the benzylamine concentration was 5 mM. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954). A representative of three replicates captured is shown. Error bars are omitted for clarity.

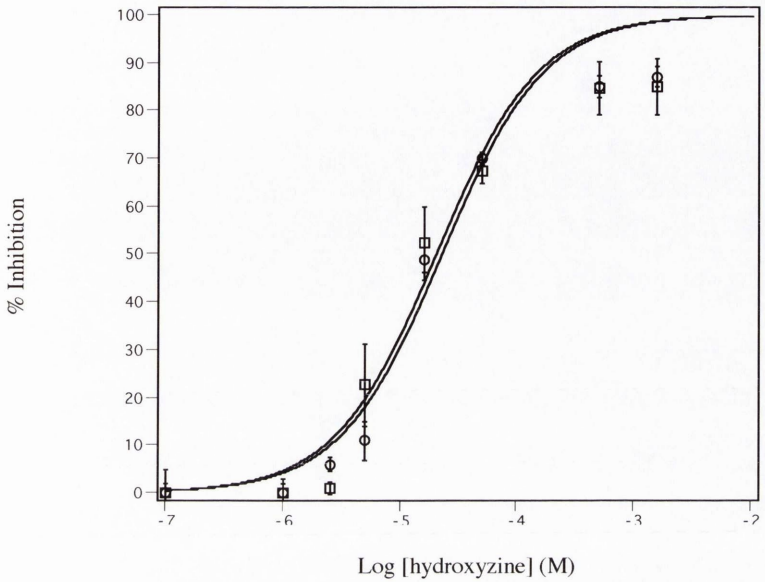


Fig. 4.8.7. The effect of incubation with hydroxyzine on the oxidative deamination of benzylamine by partially purified bovine plasma SSAO.

The enzyme (0.01 mg/ml protein) was preincubated with hydroxyzine for 5 min at 37°C before the reaction was started by the addition of benzylamine, to a final concentration of 5 mM. O = 0 min, □ = 60 min. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954) and the effect on the initial rate was determined. Each sample is the mean value ± S.E.M from triplicate determinations.

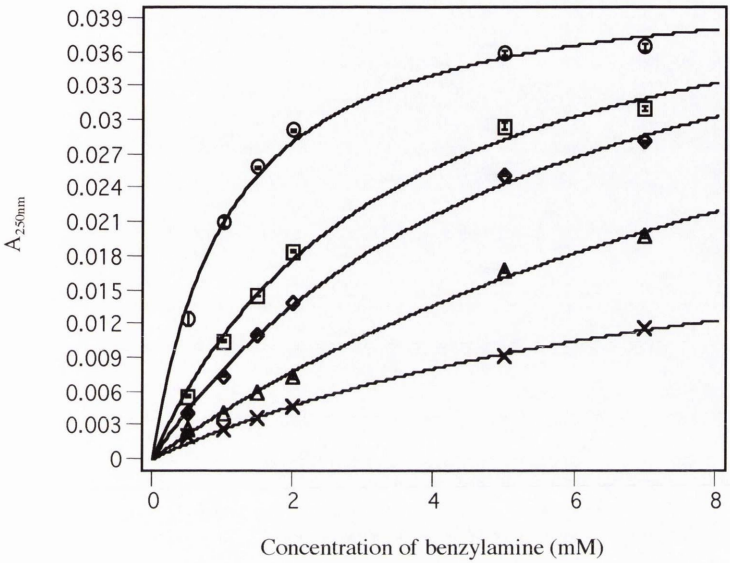


Fig. 4.8.8. The kinetics of the inhibition of partially purified plasma SSAO (0.01 mg/ml protein) by hydroxyzine.

Initial rates of reaction were determined at the indicated concentrations of benzylamine and the following fixed concentrations of hydroxyzine 0 (O), 2.5 (□), 7.5 (◇) , 10 (△) and 15 (**X**) μM. The production of benzaldehyde was determined at 250 nm, using a on a Varian Cary 300 bio spectrophotometer, by a modification (Tipton *et al.*, 2000) of the method by (Tabor *et al.*, 1954).. Each point is the mean value ± S.E.M. from triplicate determinations.

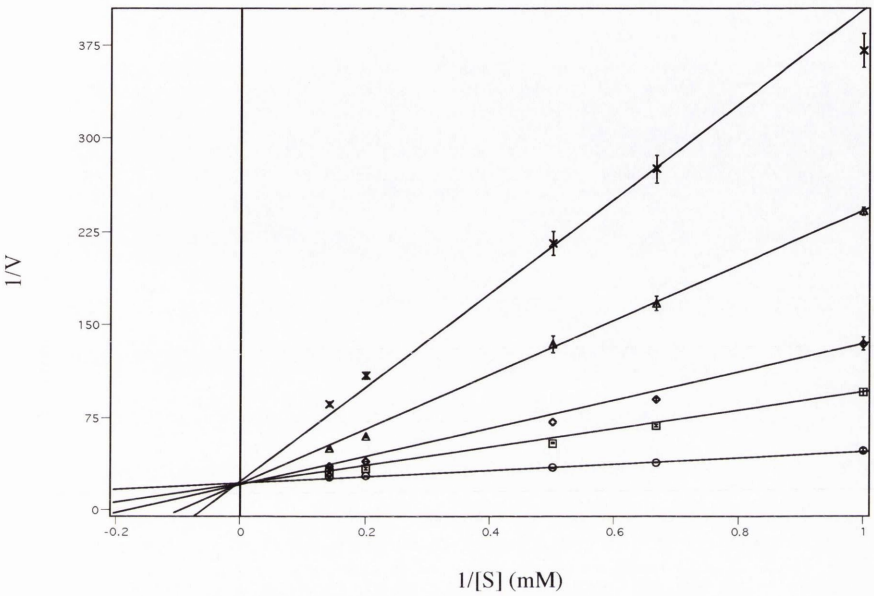


Fig. 4.8.9. Representation of the kinetics of the inhibition of partially purified bovine plasma SSAO by hydroxyzine as double-reciprocal plots..

The data from Fig. 4.8.3. are shown as a double-reciprocal plots for illustrative purposes. The concentrations of hydroxyzine were 0 (O), 2.5 (□), 7.5 (◇), 10 (△) and 15 (X) μM. Each point is the mean value ± S.E.M. from triplicate determinations. Data are shown as a double-reciprocal plot for illustrative purposes.

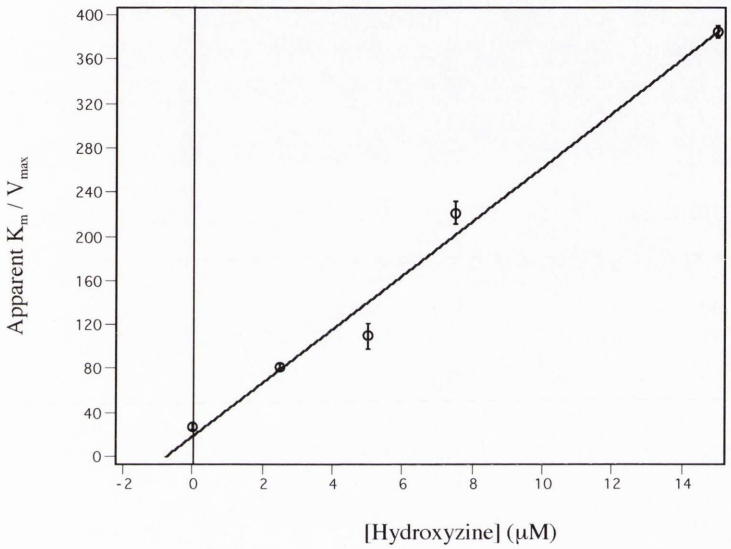


Fig. 4.8.10. Plot of the apparent values of K_m / V_{max} values determined from Fig. 4.8.8. as a function of the hydroxyzine concentration

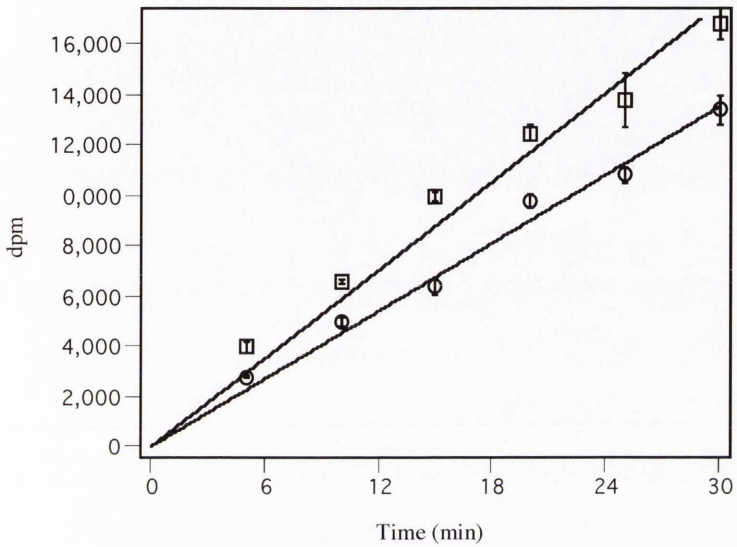


Fig. 4.8.11. Time courses of SSAO activity in bovine lung microsomes towards benzylamine.

10 µl of the microsome preparation (protein concentration 11 mg/ml) were assayed in the presence (**O**) and absence (□) of the MAO A and MAO B inhibitors clorgyline (1 µM) and deprenyl (1 µM), respectively. All samples were preincubated for 30 min at 37 ^{o}C in a shaking water bath before the reaction was initiated by the addition of ^{14}C-benzylamine (20 µM). The reaction was terminated by acidification at the times specified and the product was determined after extraction by liquid scintillation counting (Tipton *et al.*, 2000). Each sample is the mean value ± S.E.M. from triplicate determinations.

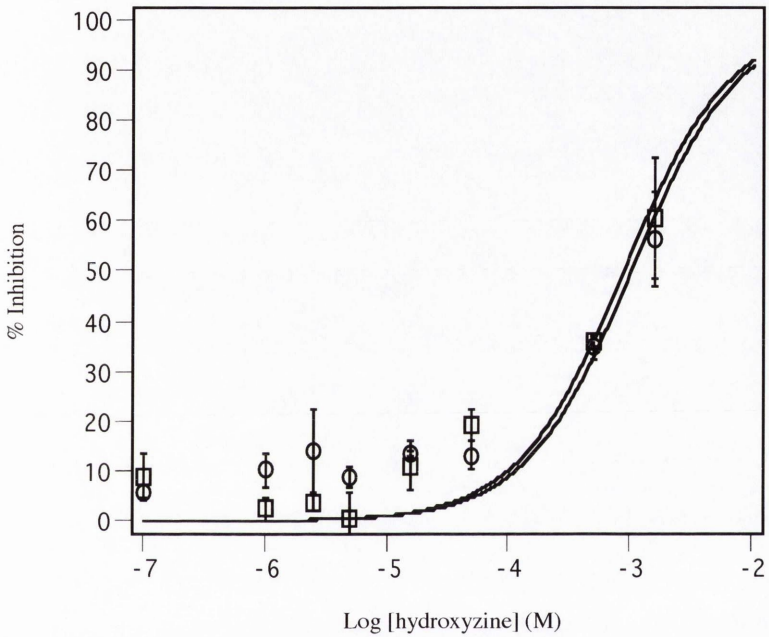


Fig. 4.8.12. The effect of time on the inhibition of membrane-bound bovine lung microsomal SSAO by hydroxyzine.

The enzyme, at a concentration was 0.49 mg/ml protein, was preincubated with hydroxyzine for 5 min at 37 ^{o}C before the reaction was started by the addition of ^{14}C benzylamine (20 μM), the reaction was allowed to progress for 10 min before termination by acidification. The product was determined after extraction by liquid scintillation counting (Tipton *et al.*, 2000). O = 0 min, □ = 60 min. Each sample is the mean value ± S.E.M. from triplicate determinations.

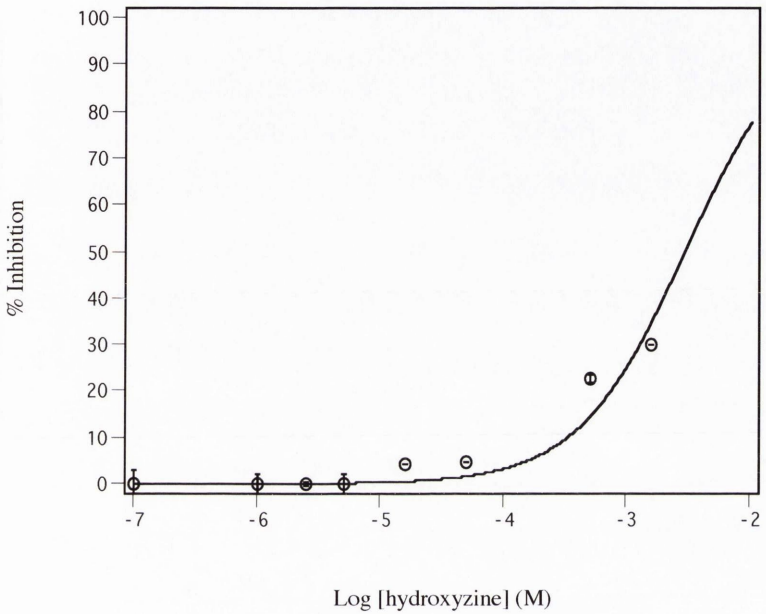


Fig. 4.8.13. The effect of incubation with incubation with endoglycosidase-H on the inhibition of microsomal membrane-bound SSAO by hydroxyzine.

The microsomal preparation (0.49 mg/ml protein) was incubated with endoglycosidase-H (1 unit) for 20 hours at 37°C before the activities towards ^{14}C benzylamine (20 μM) were determined radiochemically (Tipton *et al.*, 2000) in the presence of the indicated concentrations of hydroxyzine. Each sample is the mean value ± S.E.M. from triplicate determinations.

Fig 4.8.14. The effects of endoglycosidase-H on the inhibition of partially-purified beef plasma SSAO by hydroxyzine.

The preparation (1 mg/ml protein) was incubated with endoglycosidase-H (1 unit) for 20 hours at 37°C before the activities towards benzylamine (20 μM) were determined in the presence and absence of hydroxyzine (20μM). Activity was determined spectrophotometrically at 250nm (see Tipton *et al.*, 2000) Each sample is the mean value ± S.E.M. from triplicate determinations.

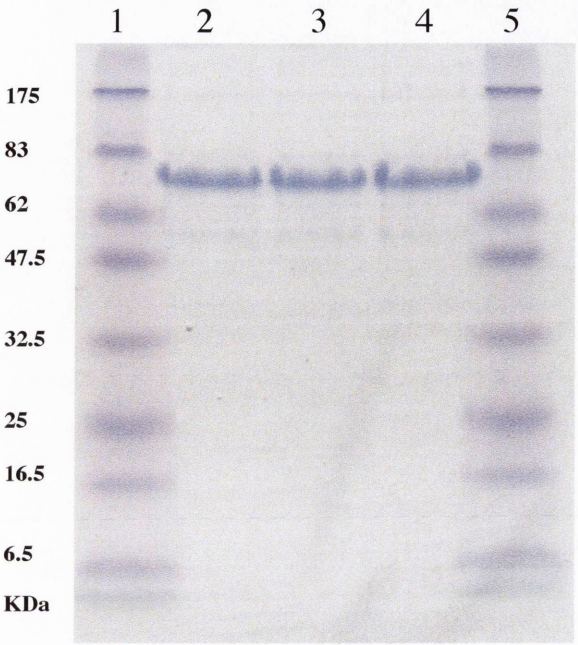


Fig. 4.8.15. SDS-polyacrylamide gel (5%) electrophoresis of partially purified bovine plasma SSAO after incubation with endoglycosidase-H.

Lanes 1 & 5: molecular weight markers. Lane 2: Control SSAO (15 μg). Lane 3: SSAO (15 μg) after 20 h incubation at 37 ^{O}C. Lane 4 SSAO (15 μg) after incubation for 20 h with endoglycosidase-H (500 mU / 200 μg SSAO). Gels were stained with Coomassie brilliant blue.

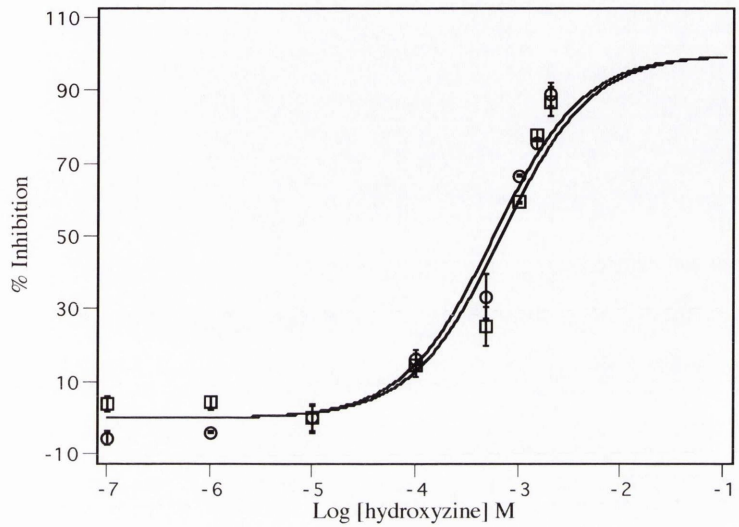


Fig. 4.8.16. The effect of incubation with hydroxyzine on the oxidative deamination of 5-hydroxytryptamine (5-HT) by rat liver mitochondrial MAO A.

The enzyme (1 mg/ml protein) was preincubated with hydroxyzine for 5 min at 37 °C before the reaction was started by the addition of ^{14}C-5-hydroxytryptamine. Product formation was determined radiochemically (Tipton *et al.*, 2000) after a reaction time of 10 min. in the presence of the indicated concentrations of hydroxyzine. O = 0 min, □ = 60 min. Each sample is the mean value ± S.E.M. from triplicate determinations.

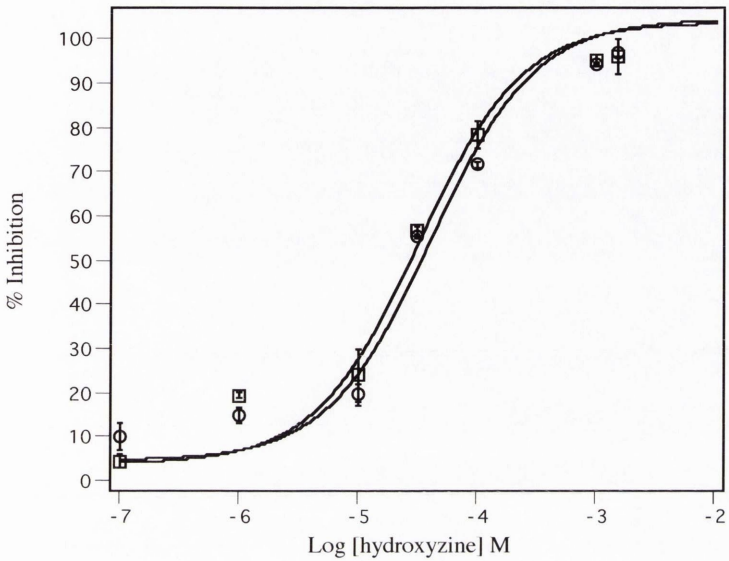


Fig. 4.8.17. The effect of incubation with hydroxyzine on the oxidative deamination of 2-phenylethylamine (PEA) by rat liver mitochondrial MAO B.

The enzyme (1 mg/ml protein) was preincubated with hydroxyzine for 5 min at 37 °C before the reaction was started by the addition of ^{14}C-2-phenylethylamine. Product formation was determined radiochemically (Tipton *et al.*, 2000) after a reaction time of 5 min. in the presence of the indicated concentrations of hydroxyzine. O = 0 min, □ = 60 min. Each sample is the mean value ± S.E.M. from triplicate determinations.

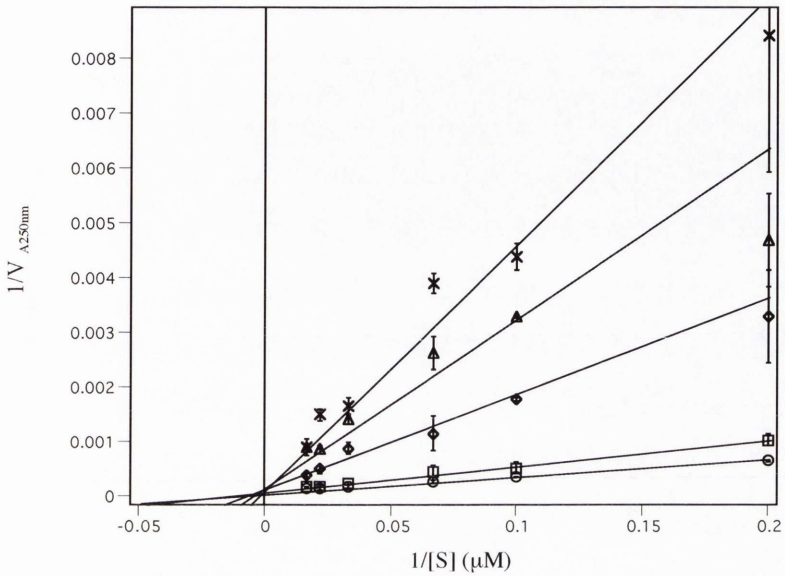


Fig. 4.8.18. The kinetics of the inhibition of rat liver mitochondrial MAO B hydroxyzine.

The enzyme (1 mg/ml protein) was preincubated with hydroxyzine for 5 min at 37°C before the reaction was started by the addition of ^{14}C-2-phenylethylamine Rates were determined radiochemically (Tipton *et al.*, 2000), after a reaction time of 5 min, at the following concentrations of hydroxyzine 0 (O), 10 (□), 100 (◇) , 175 (△) and 250 (**X**) μM. Each sample is the mean value ± S.E.M. from triplicate determinations. Data are shown as double-reciprocal plots for illustrative purposes.

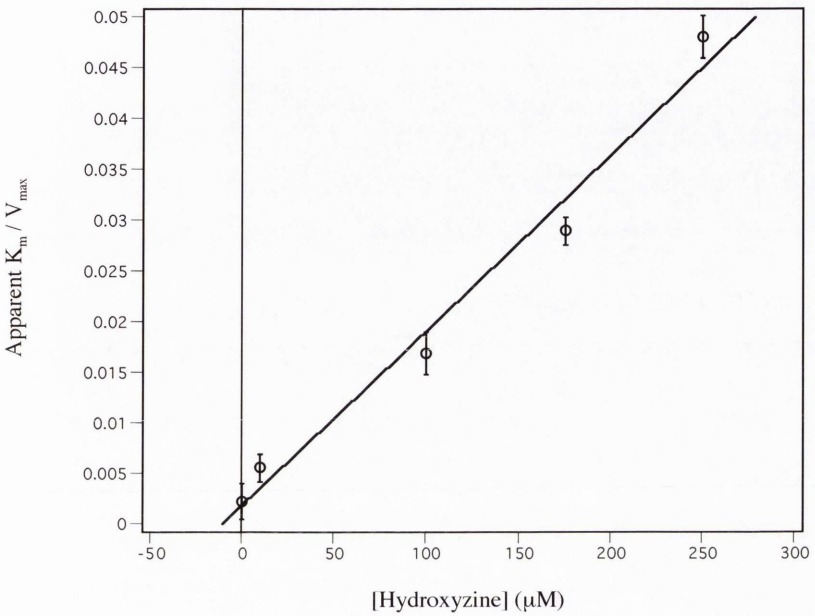


Fig. 4.8.19. Plot of the apparent values of K_m / V_{max} values determined from the data of Fig. 4.8.18. as a function of the hydroxyzine concentration

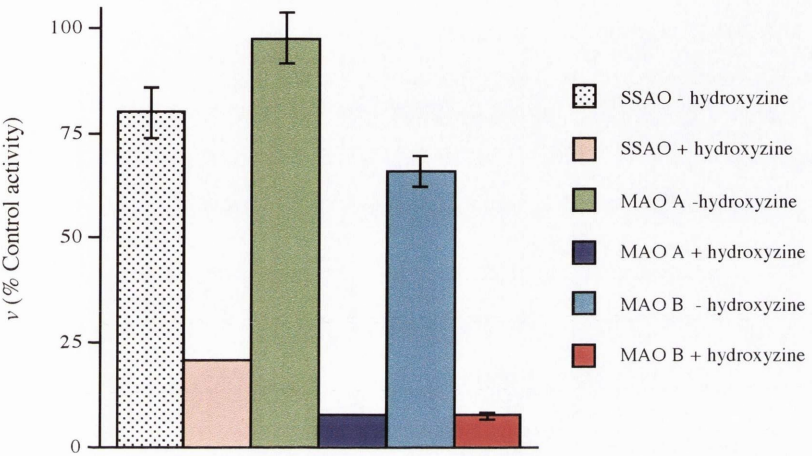


Fig. 4.8.20. Recovery of amine oxidase activity upon removal of hydroxyzine by dilution (SSAO) and centrifugation and resuspension (MAO A and MAO B)

Enzymatic assays were performed as previously described for each enzyme (Section 2.4). Each point is the mean value ± S.E.M. from triplicate determinations.

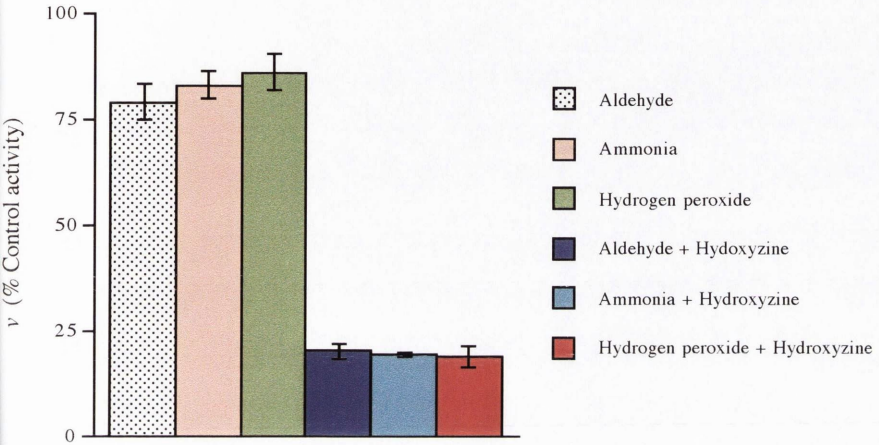


Fig. 4.8.21. The effect of the products of a MAO A catalysed reaction on the inhibition of MAO A by hydroxyzine.

The enzyme was preincubated with 5-hydroxyindole-3-acetaldehyde (1 mM) ammonia (NH_4Cl;1 mM) and hydrogen peroxide (1 mM) in the presence and absence of hydroxyzine (1 mM) for 5 min at 37 ^{O}C, in a shaking water bath, before the activity towards ^{14}C-5-hydroxytryptamine was determined radiochemically after a reaction time of 10 min (Tipton *et al.*, 2000). Each point is the mean value ± S.E.M. from triplicate determinations.

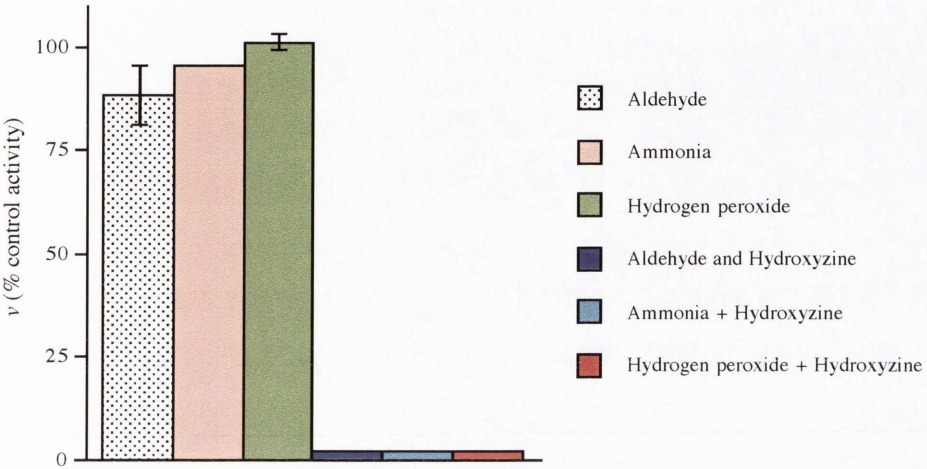


Fig. 4.8.22. The effect of the products of a MAO B catalysed reaction on the inhibition of MAO A by hydroxyzine.

The enzyme was preincubated with phenylacetyaldehyde (1 mM), ammonia (NH_4Cl;1 mM) and hydrogen peroxide (1 mM) in the presence and absence of hydroxyzine (1 mM) for 5 min at 37 ^{o}C, in a shaking water bath, before the activity towards ^{14}C-5-hydroxytryptamine was determined radiochemically after a reaction time of 10 min (Tipton *et al.*, 2000). Each point is the mean value ± S.E.M. from triplicate determinations.

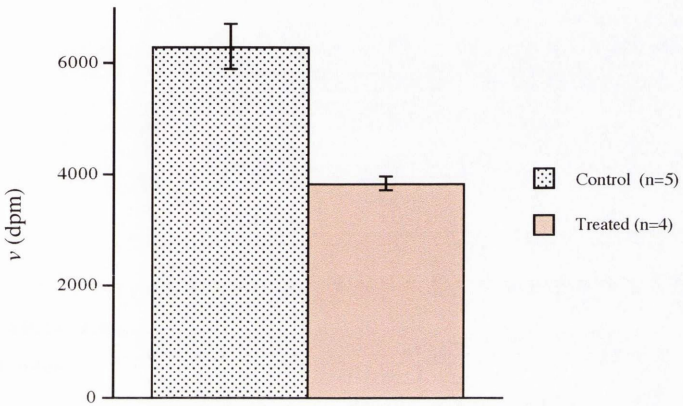


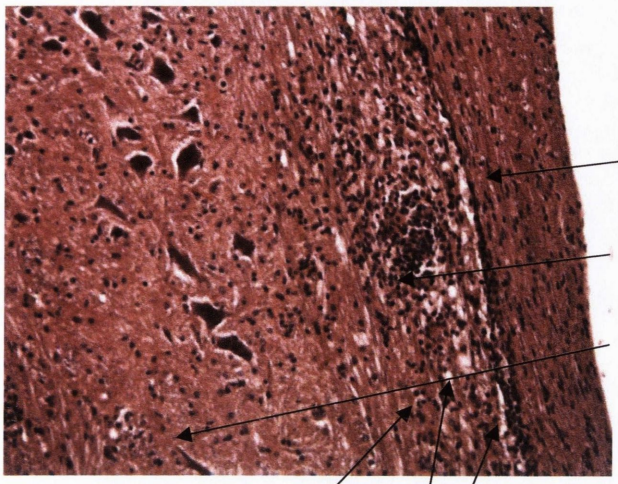
Fig. 4.8.23. The effects of 2-bromoethlyamine (10mg/kg body weight) administration on the SSAO activity in brain homogenate of EAE induced C57 black mice.

Brain homogenates (1mg/ml protein) were assayed for SSAO activity radiochemically (Tipton *et al.*, 2000) at pH 9 and after a reaction time of 10 min. Each point is the mean ± S.E.M. from triplicate determinations on each sample.

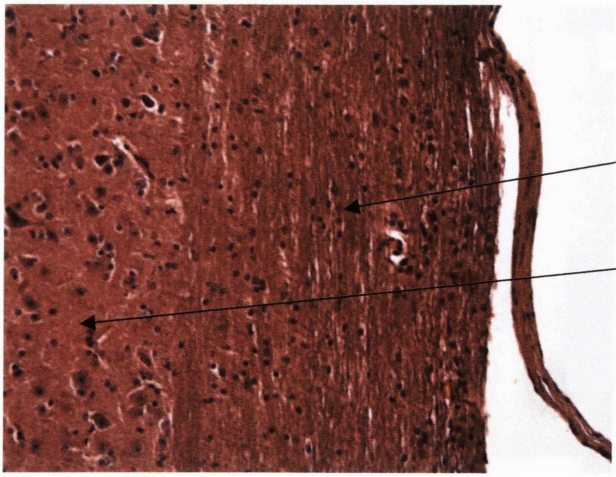
Fig. 4.8.24. Hematoxylin & eosin stained brain sections from EAE-induced C57 black mice.

30 days after induction with EAE. Treated mice received 10mg/kg body weight 2-bromoethylamine at 3 evenly-spaced intervals per week for three weeks. Magnification 200X. See text for further description.

Untreated



Treated



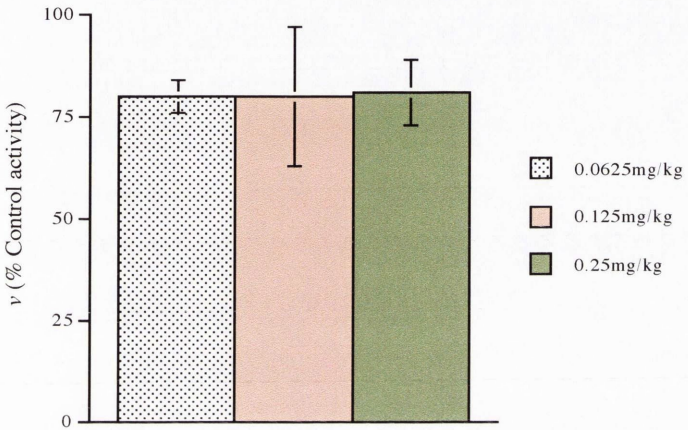


Fig. 4.8.25. SSAO activity in plasma of EAE induced C57 black mice in the presence of methylhydrazine.

The methylhydrazine concentrations were 0.0625mg/kg, 0.125mg/kg and 0.25mg/kg. Plasma samples (25 μl) were assayed for SSAO activity radiochemically (Tipton *et al.*, 2000) at pH 9. The reaction time was10 min, at 37 ^{O}C. The assay was performed in the presence of the MAO inhibitors, clorgyline plus deprenyl (each at 1 μM). Each sample is the mean ± S.E.M. of 3 replicates. N= 3 for each dose.

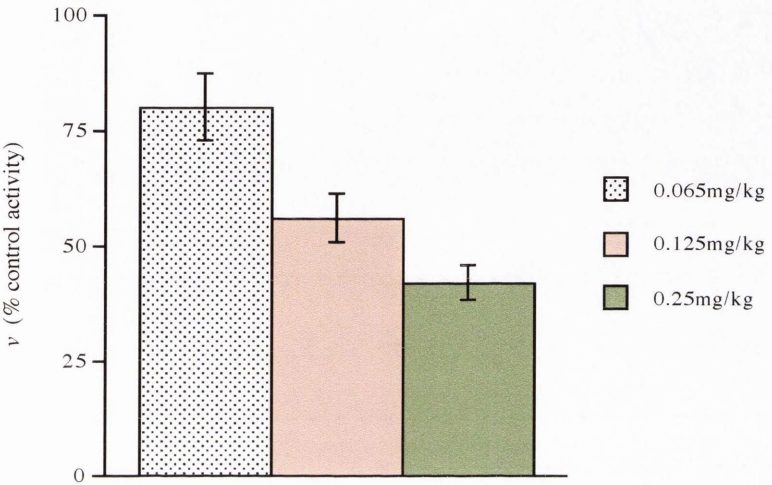


Fig. 4.8.26. SSAO activity in the lung homogenates from of EAE induced C57 black mice in the presence of methylhydrazine.

The methylhydrazine concentrations were 0.0625mg/kg, 0.125mg/kg and 0.25mg/kg. Plasma samples (25 µl) were assayed for SSAO activity radiochemically (Tipton *et al.*, 2000) at pH 9. The reaction time was10 min, at 37 ^{O}C. The assay was performed in the presence of the MAO inhibitors, clorgyline plus deprenyl (each at 1 µM). Each sample is the mean ± S.E.M. of 3 replicates. N= 3 for each dose.

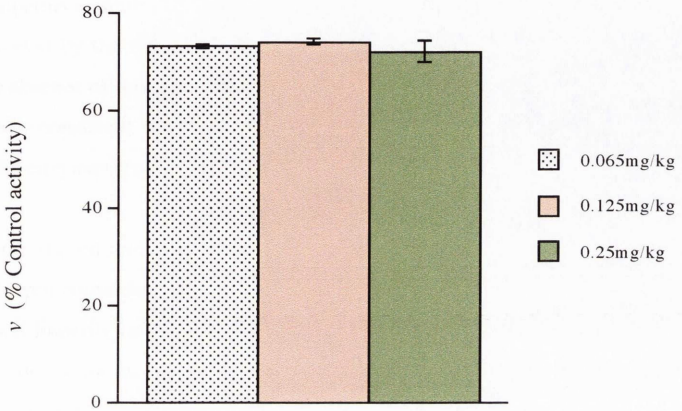


Fig. 4.8.27. SSAO activity in the brain homogenates from of EAE induced C57 black mice in the presence of methylhydrazine.

The methylhydrazine concentrations were 0.0625mg/kg, 0.125mg/kg and 0.25mg/kg. Plasma samples (25 μl) were assayed for SSAO activity radiochemically (Tipton *et al.*, 2000) at pH 9. The reaction time was10 min, at 37 ^{O}C. The assay was performed in the presence of the MAO inhibitors, clorgyline plus deprenyl (each at 1 μM). Each sample is the mean ± S.E.M. of 3 replicates. N= 3 for each dose.

4.9 Discussion

Competitive inhibition of bovine plasma SSAO by hydroxyzine was reversible, as indicated by the recovery of activity following dilution of an enzyme-inhibitor mixture. The absence of any time-dependent change in inhibitory potency during inhibition would also be consistent with reversibility. Hydroxyzine was found not to be a substrate for any of the enzymes studied.

Unlike the inhibition of SSAO by hexosamines the inhibition was not affected by the hydrogen peroxide generated in the reaction. Such behaviour would be expected to result in non-linearity in the reaction progress curve for benzylamine oxidation in the presence of hydroxyzine as a result of H_2O_2 formation. This was not the case. Furthermore, this conclusion was confirmed by the observation that the presence of catalase had no effect on the degree of inhibition.

Comparisons between commercially available bovine plasma SSAO and partially purified bovine plasma SSAO, prepared in the course of this work, showed a similar inhibition pattern with kinetic constants showing little difference. However inhibition of membrane bound was found to be significantly less sensitive to inhibition, (IC_{50} values of ~20 μM for plasma SSAO compared to 1 mM for the membrane bound form using benzylamine as a substrate). The membrane location may play a role in this difference and Lizcano *et al.* (1998) have also reported inhibitor-sensitivity differences between membrane-bound and plasma SSAO. Since SSAO is a heavily glycosylated protein, differences in glycosylation between membrane-bound SSAO and the plasma form, which is believed to originate due to cleavage of the membrane form (Kurkijärvi *et al.*, 1998), might be involved. In order to test this, endoglycosidase-H, a glycosidase with a relative broad range in terms of carbohydrate cleavage, was preincubated with membrane-bound SSAO in an effort to deplete the carbohydrate moieties on the enzyme. Incubation of microsomes with the glycosidase for 20 hours did not affect enzyme activity. However, it decreased the sensitivity to inhibition by hydroxyzine. Thus although the glycosylation of SSAO may affect the accessibility of hydroxyzine it would

appear to enhance, rather than impede, this. As microsomes were the source of the membrane-bound form of SSAO, it was not possible to determine the degree to which deglycosylation of the enzyme itself had occurred. SSAO has been shown to bind amiloride and some other imidazoline and guanidine compounds (Novotny *et al.*, 1994). However, it is not known whether this 'amiloride-binding protein' function significantly reduces the pharmacological actions of such compounds as an inhibitors of Na^+-transporting systems, and diuretic and hypertensive agents (see Frelin *et al.*, 1987). Amiloride and related compounds have also been shown to inhibit SSAO (see Mu *et al.*, 1994) although the sensitivity varies considerably between species and tissues (Lizcano *et al.*, 1998). The K_i value of inhibition of bovine plasma SSAO by amiloride (196 μM; Mu *et al.*, 1994) is somewhat higher than that reported here for inhibition by hydroxyzine. Further work would be necessary to show whether or not this binding significantly affects the function of hydroxyzine as a histamine-1 receptor antagonist *in vivo*. Since SSAO also reversibly binds a number of transport-inhibiting antidepressants, such as imipramine, maprotiline, nomifensine and zimeldine (Obata and Yamanaka, 2000), it appears that a drug-binding function should be added to the repertoire of activities of this enzyme. Because of the complexity of SSAO functions the effects of drugs that extraneously inhibit its activity may, themselves be complex.

As with SSAO, MAO activity was inhibited in a freely reversible and competitive manner by hydroxyzine. The manner of inhibition was not affected by the presence of any of the end products generated from the reaction. MAO B was inhibited significantly more than MAO A and at a comparable level to that of SSAO inhibition by hydroxyzine, which may reflect the similarity of their preference for the substrate benzylamine.

The reduction observed in mast cell activation by hydroxyzine treatment in C57 black mice induced with experimental allergic encephalomyelitis (EAE) suggested a possible role for SSAO in the progression of EAE. Since this is an inflammatory disease, which is reliant on the migration of inflammatory cells into the brain, the vascular-adhesion function of SSAO may be involved. The suicide inhibitor of SSAO, 2-bromoethylamine (Yu *et al.*, 2001), was shown to decrease the SSAO activity after administration to C57

black mice. Histological analysis of brain tissue from both treated and untreated mice showed a marked difference in the progression of EAE. Determination of the advancement in EAE in the mice is through mobility observations, the brain sample used was from a mouse exhibiting hind limb paralysis, which is indicative of late onset EAE (Furlan *et al.*, 2001). It appeared through histological comparison that treatment with 2-bromoethylamine was effective in reducing the onset of EAE by markedly decreasing the volume of inflammatory cell infiltrate in conjunction with a significant inhibition of SSAO. However, 2-bromoethylamine is a relatively weak SSAO inhibitor (see Tabata *et al.*, 2003) with an intrinsic reactivity that may result in other interactions within the tissues. Therefore, the effects of another SSAO inhibitor were investigated for comparison. Methylhydrazine has been reported to be a highly potent SSAO inhibitor, with a reported IC_{50} value of 7 nM (Holt *et al.*, 1992). However, there was an unexpected high mortality rate in the mice treated with this compound. This may be a specific response of in the C57 black mouse strain used for the present work. However, it prevented a thorough examination of any effects that methylhydrazine may have on the progression of EAE in this model. When tissue and plasma samples were recovered the SSAO activity was found to be reduced, although in brain tissue and plasma no concentration dependent effect were observed, suggesting a possible saturation of the drug in these samples. In contrast, lung tissue, which contains significantly higher SSAO activity than the other samples recovered, showed a concentration dependent-inactivation of SSAO. Histological examination of brain recovered from the mice that had been treated with methylhydrazine showed no evidence for prevention of the onset of EAE. Methylhydrazine is an freely reversible inhibitor of SSAO (Holt *et al.*, 1992), which may in part explain the ineffectiveness of it in preventing EAE. Furthermore, it is unlikely that this compound shows great selectivity towards SSAO. Indeed it has been reported to cause an increase of MAO activity (Holt *et al.*, 1995), which may result in an increase in MAO-derived amine metabolites. Since many of these are toxic, this may have contributed to the mortality rate displayed by the mice in this study.

Thus these studies suggest that decreasing SSAO activity might result in a reduction in the onset of EAE. The toxicity of methylhydrazine makes it unsuitable for further studies

with this strain of mouse. Further work should focus on the search for other, less toxic means of reducing SSAO activity. Clearly, because of its relatively low potency and alternative action as a histamine-1 receptor antagonist hydroxyzine would be unsuitable for this purpose. Examination of a broader range of SSAO inhibitors may reveal a more suitable drug, which might be administered in conjunction with MAO inhibitors in order to alleviate any possible effects arising from potentiation of MAO activity. The application of specific SSAO-directed RNAis might also yield valuable insights.

This study does, however, widen the range of activities of SSAO in both drug-binding capabilities and as a pathologically significant entity. The further knowledge regarding MAO inhibition displayed can also have implications for various treatment regimes, since hydroxyzine is a commonly-prescribed drug, this may result in unforeseen complications if prescribed in conjunction with MAO inhibitors.

Chapter 5

Metabolism of endogenous amine oxidase substrates and their role in glucose uptake by adipocytes

5.1 Introduction

5.1 Glucose metabolism

The well-established roles of glucose in biological systems are summarized in Fig. 5.1. As glucose, either directly or derived from other carbohydrate compounds such as starch, is the major source of energy it is essential that the transport, storage and metabolism be highly controlled and regulated. Carbohydrate absorption occurs mainly at the small intestinal brush border. Glucose is absorbed mainly in the jejunum by an active process, whereby it enters cells via a sodium-dependent co-transporter protein on the apical side of the enterocyte. which are cells of the intestinal epithelium. Sodium ions pass down electrochemical gradient, into cells to replace sodium ions which are actively being transported out of cell on basolateral membrane by Na^+/K^+ ATPase pumps. Glucose diffuses out of the cell into intercellular space and from there into local capillaries. Chloride ions and water accompany the movement of sodium and glucose (Wright *et al.*, 2003).

Once in the cell glucose may be used for energy production via the glycolytic pathway, citric acid and the electron transport chain. The chemical energy within the glucose molecule is eventually released in the form of ATP through the ATP synthase (complex V of the respiration chain) along with two ATPs from glycolysis. One glucose molecule can give rise to a net gain of 36 molecules of ATP. ATP can then be used to drive numerous biological reactions. Alternatively glucose can be stored in a readily-accessible form in the liver and in muscle if there is an excess, by converting it to glycogen. Gluconeogenesis is the mechanism whereby if a shortfall in glucose has occurred, the body can synthesise the compound from non carbohydrate sources of carbon such as protein.

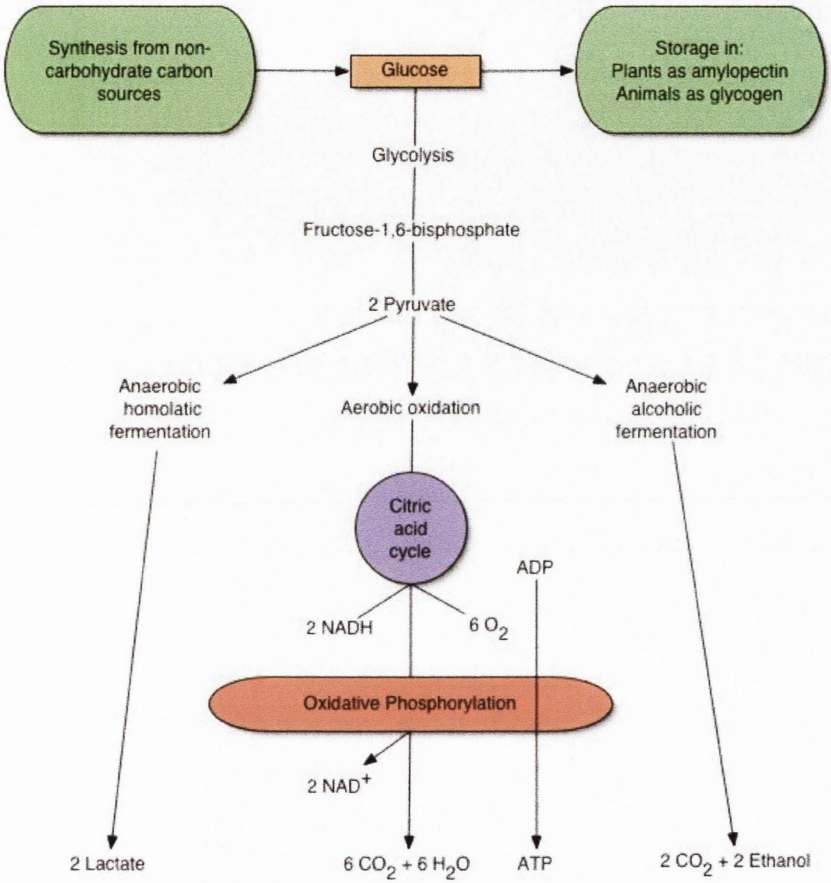


Fig 5.1 Brief outline of glucose metabolism. Modified from Voet & Voet.

5.2 Glucose in health

As glucose is an integral part of mammalian cell function, including an involvement in concert with other nutrients, in regulation of fundamental cellular processes such as cell cycle progression (Bohnsack *et al.*, 2004) any dysfunction in the metabolism of the

hexose would result in numerous complications. Outlined within this section are several of the main disease states associated with an imbalance in the regulation and metabolism of glucose.

5.2.1 Diabetes Mellitus

Diabetes mellitus is probably the best known of the disease states involving glucose, although in diabetes the problem lies with insulin. Insulin is a polypeptide hormone, which acts mainly on muscle and adipose tissue to stimulate the synthesis of glycogen, fats and proteins while inhibiting the breakdown of these metabolic fuels. In addition to this role insulin will also stimulate the uptake of glucose into most tissues, with the exception of liver and brain. In diabetes insulin is either not expressed in sufficient quantities or does not stimulate target cells efficiently. Both these states lead to cell starvation, as glucose uptake into cells is impaired, and blood glucose levels are elevated. The tissues respond to this apparent starvation by triacylglycerol hydrolysis, fatty acid oxidation, gluconeogenesis and ketone body generation. As ketone body production increases, their acidic nature causes a strain on the bloods buffering capacity. In addition the kidneys (which maintain blood pH) must excrete excess H^+ accompanied by K^+, P_i and H_2O which exacerbates the osmotic effect of the high glucose resulting in severe dehydration. This condition, referred to as ketosis, can be fatal.

There are two main forms of diabetes Type I, or insulin-dependent, and Type II, noninsulin dependent. Type I is due to an absence of insulin because the pancreas lacks or has defective β cells. In genetically susceptible individuals β cells are destroyed by an autoimmune response which slowly, over years, reduces β cells numbers. At around 80% reduction in β cell numbers classic diabetic symptoms emerge. Type II diabetes accounts for 90% of diabetic cases and usually occurs in obese individuals with a genetic predisposition to the disease. Although patients with type II diabetes have at least normal, and in some cases abundant, insulin levels, the disease results from a lack of insulin mediated glucose uptake due to insulin receptor insensitivity at the target-cell surface. The current hypothesis suggests that overeating results in a suppression of insulin

receptor expression and it has been shown that diet alone can help control type II diabetes.

There are two other but less well known forms of diabetes, gestational diabetes and pre-diabetes. Pregnant women who have never had diabetes but who have high blood sugar (glucose) levels during pregnancy are said to have gestational diabetes. This form of diabetes arises from a conflict between mother and child, as the foetus develops, hormones from the placenta aid in its progression but they may also cause insulin resistance in the mother resulting in hyperglycemia. Pre-diabetes is a stage before type II diabetes where patients have high glucose levels yet do not show symptoms of type II diabetes. Even if maintained at this level a patient will experience eventual damage to the heart and circulatory system.

5.2.2 SSAO and Diabetes

A role of SSAO in diabetes was first indicated by elevated SSAO levels in plasma in diabetic subjects (Lewinsohn, 1984) and again in studies with streptozotocin-induced diabetic rats. Streptozotocin-induced diabetic rats are a commonly-used animal model for Type I diabetes. SSAO activity was determined from a number of tissues in the animals and was found to be selectively increased in both serum and kidneys (Hayes *et al.*, 1990). Increased levels of methylamine and SSAO, which are also found in several other pathological states, can lead to the over production of formaldehyde (Boor *et al.*, 1992). Although methylamine is relatively harmless to endothelial cells formaldehyde is believed to result in cytotoxic damage in endothelial cells. As both SSAO and methylamine levels are increased in diabetes it is possible that these are the factors that induce the atherosclerotic plaque formation in the cardiovascular disorders seen in diabetes (Yu *et al.*, 1993). Methylglyoxal, which is derived from the SSAO-catalysed oxidative deamination of aminoacetone (Deng *et al.*, 1999), has also been implicated in the vascular problems associated with diabetes, since it enhances advanced glycation end product (AGE) formation by its interaction with proteins (Mathys *et al.*, 2002). Inhibition of SSAO (by irreversible inhibitors such as aminoguanidine and hydralazine) was shown to alleviate these aldehyde-induced problems, at least to some extent these studies

showed a significant decrease in AGE and aldehyde adducts (Yu *et al.*, 1997, Gronvall *et al.*, 1998). Congestive heart failure (CHF) is a condition commonly associated with diabetes and vascular endothelial dysfunction is associated with both types. Patient studies with diabetic patients have demonstrated a correlation between the severity of CHF and increasing SSAO activity (Boomsma *et al.*, 1997). This again suggests a situation, similar to those previously discussed, where the cytotoxic products of amine oxidation can cause, or increase, the vascular damage associated with these diseases.

Elevated SSAO levels were originally observed in Type I diabetics and subsequently also shown also to occur in Type II diabetics (Garpenstrand *et al.*, 1999, Meszaros *et al.*, 1999). Both of these studies revealed a link between the development of other diabetic symptoms, such as retinopathy and angiopathy as well as atherosclerosis, and the elevated levels of SSAO, through the production of toxic metabolites. A long-term study has confirmed the relationship between the levels of SSAO and the development of diabetic retinopathy (Gronvall-Nordquist *et al.*, 2001).

Although increased SSAO activity appears to be responsible for a number of the complications found in diabetics, a beneficial side has also emerged. When strepozotocin induced diabetic rats were treated with the non-physiological SSAO substrate benzylamine in the presence of low concentrations of vanadate a reduction in hyperglycemia was observed. Glucose transport was stimulated in isolated adipocytes with resultant antidiabetic effects (Marti *et al.*, 2001). The insulin-like effect exerted by SSAO results from stimulation of the GLUT-4 glucose transporter recruitment to the cell surface. Tyrosine phosphorylation of both insulin receptor substrates 1 and 3 (IRS-1, IRS-3) and phosphatidylinositol 3-kinase (PI3K) was stimulated by the presence of the SSAO substrates benzylamine and tyramine, substrates which are also oxidised by MAO (Zorzano *et al.*, 2003). Similar results were observed in Goto-Kakizaki rats, which are a model for type II diabetes (Abella *et al.*, 2003a). Mechanistically it would appear that peroxyvanadium is responsible for the antidiabetic effect, since it stimulate insulin secretion in pancreatic islets and activating glucose metabolism in adipocytes (Abella *et al.*, 2003b). However vanadate is not a requirment in all systems (Morin *et al.*, 2002). An alternative Type II diabetic model, the KKay mouse, showed a reduction in glucose transport when treated with the highly potent and selective SSAO inhibitor SSAO

inhibitor E0-2-(4-fluorophenethyl)-3-fluoroallylamine (FPFA) and this was accompanied by a decrease in the number of atherosclerotic lesions, further suggesting a significant role for SSAO in diabetes (Yu *et al.*, 2004).

The increased SSAO activity in diabetes and its origin is still unclear. Originally an up regulation in SSAO gene expression was believed to be responsible for the increased activity but when NMRI mice received an injection of alloxan, which induces diabetes, the mRNA levels in adipose tissue were actually reduced possibly, due to negative feedback, although SSAO activity was increased (Nordquist *et al.*, 2002). This negative feedback phenomena was observed in a transgenic mouse (NMRI model) overexpressing SSAO, which it was suggested might result from a post-translational modification of existing SSAO molecules or the activating of a previous inactive SSAO molecule (Gokturk *et al.*, 2004).

5.2.3 Diabetes and Insulin in Alzheimer's Disease (AD)

Changes in blood glucose (lower) and insulin (higher) levels in patients with senile dementia of Alzheimers type (SDAT) was recognized in the early 1980s but it was found that these patients did not suffer from diabetes (Bucht *et al.*, 1983). Insulin regulates phosphorylation of tau protein, which is implicated in the neurofibrillary lesions in the brains of AD patients, and also affects the metabolism of beta-amyloid, the main constituent of AD amyloid pathology. No correlation was found with polymorphisms in the 5' end of insulin gene, which had been associated with blood glucose levels, and AD (Fisman *et al.*, 1986). Reduced glucose metabolism has also been reported in patients with AD and is believed to be due, in part, to alterations in physical activity (Meneilly *et al.*, 1993). This results in an increase in release of cerebral amino acids (Hoyer *et al.*, 1989). Further studies have shown that desensitization of the neuronal insulin receptor, an early event in SDAT, causes disturbances in glucose metabolism (Hoyer 1996).

Although the question of whether or not diabetes is associated with dementia has been frequently asked, the answers have been relatively vague and contradictory. However a study in Rotterdam suggested that Type II diabetes is associated with dementia and that AD may be more frequent in elderly diabetic patients treated with insulin (Ott *et al.*,

1996). Further evidence points to genetic variance of the apolipoprotein E genotype as risk factor for AD. Patients without an epsilon-4 allele had significantly lower insulin levels, thereby further reducing their metabolic capacity and exacerbating their condition (Craft *et al.*, 1999a). In view of this reduced insulin functionality, it would seem that an obvious course of treatment would be administration of insulin and this has appeared successful, by raising the availability of glucose, in patients not suffering from hyperglycemia (Craft *et al.*, 1999b). It would appear that the increase in the upregulation of insulin receptors is a compensatory mechanism by which the reduced levels of glucose metabolism can be somewhat overcome (Frolich *et al.*, 1999). The previously mentioned studies suggested a decrease in insulin efficiency even with a detected increase in insulin receptor expression and a suspected inhibition was, therefore, examined. Amyloid- beta peptide is a associated with the pathology of AD and is similar to insulin as both are amyloidgenic peptides with a common sequence recognition motif and both are substrates for the same insulin degrading enzyme. Competition studies did, indeed, show that amyloid-beta is a competitive inhibitor of insulin binding and, hence, insulin action (Xie *et al.*, 2002). Glucagon-like peptide-1 (GLP-1) is an insulinotropic hormone that stimulates pancreatic islet beta-cell proliferation and glucose dependent insulin secretion, lowering blood glucose in patients suffering from type II diabetes. Recent studies have shown that GLP-1 also dose-dependently reduces amyloid-beta peptide levels in the brain (Perry *et al.*, 2002) and protects against oxidative injury (Perry *et al.*, 2003). Thus this peptide appears to have potential as a therapeutic target for intervention in AD.

Although this section focused on the role of glucose metabolism in Alzheimer's disease and the link with diabetes, many other neurodegenerative disorders are associated with diabetes, including ataxia-telangiectasia, Down's syndrome/trisomy 21, Friedreich's ataxia and Huntington's disease. (see Ristow 2004).

5.2.4 Glycogen storage disease

The glycogen synthesis and degradation pathways involve a set of enzymes whose failure can lead to a group of diseases collectively known as glycogen storage diseases (GSD). The enzymes are found in cytoplasm, endoplasmic reticulum and lysosomes and many are specific to individual organs. Twelve types of glycogen storage diseases have been identified (Hug 1976) and are caused by inherited deficiencies of the enzymes involved in the regulation of synthesis or degradation of glycogen. A strict dietary regime can relieve several symptoms, such as hyperglycemia and may decrease the size of the liver, resulting in normal, or close to normal, development. GSD related to the lack of phosphorylase activity are generally milder and the symptoms disappear by puberty (Wolfsdorf *et al.*, 1999).

5.2.5 Glucose-galactose malabsorption

Glucose–galactose malabsorbtion is an autosomal recessive disease that presents in newborn infants on the ingestion of glucose, lactose and galactose as a life-threatening diarrhea. The disease is caused by a deficiency of the secondary active sodium/glucose transporter (Brown 2000) due to mutations on the sodium-glucose cotransporter gene (SGLT1) (Wright *et al.*, 2002). Initial treatment to overcome the disease is based on dietary modification, which consists of the removal of glucose, lactose and galactose until the acute diarrheic phase is controlled, followed by the replacement of glucose and galactose in the diet with fructose (Pascual *et al.*, 2004), which does not have any known defects in its absorption (Wright *et al.*, 2003). Lactase deficiency is common in adults affecting mainly Asian, African Americans, Native Americans and Mediterranean populations and is due to a deficieny of lactase in the intestine. Lactase is responsible for the breakown of lactose which is a component of milk and dairy products.

5.2.6 Syndrome X

Syndrome X is an impairment of glucose tolerance linked to coronary heart disease (CHD). Along with insulin resistance, in respect to glucose uptake, hypertension, obesity and dyslipoproteinemia all add to an increased risk of CHD (Beck-Nielsen *et al.*,1990). The mechanism behind the onset of Syndrome X is unclear, but plasminogen activator inhibitor-1, which is an adipocytokine, was shown to be overproduced in visceral fat tissue in patients and may play a role in the development of the disorders in Syndrome X (Matsuzawa *et al.*, 1999). More recent studies though have shown that the insulin:glucagon regulation of blood glucose levels can be complemented by growth hormone and in essence describes Syndrome X as an incipient type II diabetes in which the glucose control system, while impaired, can still maintain normal blood glucose levels (Koeslag *et al.*, 2003).

5.3 Glucose transport and glucose transporters

5.3.1 Glucose transport

There are three recognised mechanisms of glucose transport into the cell, the first is the sodium-glucose co-transport which relies on a membrane protein family known as SGLT. This family of proteins catalyse the uphill movement of glucose using the driving power of a downhill movement of sodium ions (Wright 2001). A mechanism of glucose transport unique to bacteria known as group-translocation utilises the phosphoeynolpyruvate phosphotransferase system (PTS). Along with the movement of glucose across the membrane it allows the phosphorylation of the glucose molecule, maintaining a glucose concentration gradient across the membrane and producing an intracellular accumulation of glucose phosphate (Meadow *et al.*, 1990).

The mechanism of glucose transport studied in this work is the facilitated or carrier-mediated glucose transport. Facilitated transport across a biological membrane relies on specific transporter proteins within the membrane. The molecule to be transported across the membrane binds the transport protein on one side of the membrane, this binding

instigates a conformational change in the protein allowing transport across the membrane and release of the molecule on the other side. A well studied example of this type of transport is the erythrocyte glucose transporter (GLUT1). The mechanism of this and other forms of GLUT proteins will be considered in the next section

5.3.2 Glucose transporter proteins (GLUT)

Facilitated transport of glucose across the membrane utilises a family of transmembrane proteins known as Glucose Transporter Proteins (GLUT). Originally GLUT 1 was isolated and cloned (Mueckler *et al.*, 1985) and, since then, many other members of the family have been identified. At present 13 members of the family have been recognised and are designated GLUT 1-12 with the thirteenth named HMIT (H^+-coupled myo-inositol transporter). It is believed that all members have now been documented. Based on sequence similarities GLUTs have been further subdivided into 3 subclasses (I-II & III) (Joost & Thorens 2001). Structurally GLUTs consist of 12 membrane spanning domains (Mueckler *et al.*, 1985) some of which line a water filled glucose pathway and this is similar for all the isoforms (Mueckler *et al.*, 1985). Another structural feature consistent in all isoforms of the transporter is a glycosylated loop between transmembrane helices 1 and 2, this feature is believed to play a role in the tissue specific regulation of the transporter function, as each isoform possesses a significantly different and diverse amino acid sequences at this point (James 1994). See table 5.1 for the tissue distribution of GLUTs.

Isoform	Class	Main tissue localisation
GLUT 1	I	Erythrocytes, brain, ubiquitous
GLUT 2	I	Liver, pancreas, intestine, kidney
GLUT 3	I	Brain
GLUT 4	I	Heart, muscle, WAT, BAT, brain
GLUT 5	II	Intestine, testes, kidney
GLUT 6	III	Brain, spleen, leucocytes
GLUT 7	II	n.d.
GLUT 8	III	Testes, brain and other tissues
GLUT 9	II	Liver, kidney
GLUT 10	III	Liver, pancreas
GLUT 11	II	Heart, muscle
GLUT 12	III	Heart, prostate, muscle, small intestine, WAT
HMIT	III	Brain

Table 5.1 Tissue distribution of different GLUT isoforms.Modified from Wood & Trayhurn 2003.

5.3.3 Regulation of GLUT function

The most widely studied mechanism through which the GLUTs are regulated is the control exerted on GLUT 4 by insulin, which is generally mediated through the phosphatidylinositol-3-kinase (PI3K) pathway (Lee *et al.*,1995). Recruitment of GLUT 4 occurs via the PI3K pathway although not as a direct result of PI3K activation but using PI3K as an intermediate in the activation of the protein kinase Bb/Akt 2 (Isakoff *et al.*,

1995). Akt 2 phosphorylates vesicular proteins including GLUT 4 thereby causing the dissociation of the vesicles from an intracellular anchor, resulting in fusion with the plasma membrane (Kupriyanova & Kandror 1999). Further pathways involving insulin stimulation exist, as the activation of Akt has been deemed insufficient for insulin stimulated glucose uptake alone (Imanaka *et al.,* 1998). Such pathways include the involvement of a GTP-binding proteins G(q) and/or G(11), which appear to stimulate insulin mediated GLUT 4 translocation in a PI3K-independent manner (Kanzaki *et al* 2000). In studies in mice G(11) translocation of GLUT 4 was blocked by the expression of a dominant inhibitory form of the actin-regulatory GTPase, ARF6 but only under stimulation by endothelin and not through insulin signalling pathways involved in GLUT 4 translocation (Bose *et al* 2001). Although the endothelin pathway appears to parallel the insulin one, at least up to a point, the processes stimulate functionally different pools of glucose transporters (Lawrence & Birnbaum 2001). The insulin responsive storage pool of GLUT 4 vesicles are the usual target during insulin stimulation but translocation can also occur from the recycling endosomal pool which also contains GLUT 1 (Ducluzeau *et al.,* 2002). Targeting between these distinct compartments is microtubule dependent and disruption of microtubules can direct the signalling traffic to recruit the transporters from an alternative GLUT pool (Liu *et al.,* 2003).

Osmotic shock regulation of GLUT 4 translocation was believed to utilise the same pathway as insulin but it has been shown to be mediated via a separate novel tyrosine kinase pathway (Chen *et al.,*1997). In addition to effecting this alternative pathway to insulin signalling, osmotic shock totally inhibits the insulin signalling pathway by maintaining Akt in an active dephosphorylated state (Chen *et al.,* 1999). The end result of the osmotic response appears to affect the same target as the insulin pathway mediated by another insulin receptor substrate, Gab-1 (Janez *et al* 2000). After bypassing the initial insulin signal transduction steps, the pathways converge downstream of and/or in parallel to the PI3K/Akt pathway (Kralik *et al.,* 2002).

Alternative regulatory factors have also been found to exist in several dysfunctional states such as ischemia and hypoxia. Regulation of GLUT 1 gene expression is partially mediated by adenosine A2R receptors and the cAMP-PKA pathway in hypoxia in retinal endothelial cells, resulting in an increase in glucose uptake (Takagi *et al* 1998). AMP

activated protein kinase (AMPK) has a role in GLUT 4 translocation, with a contributory role from the NO-guanylate cyclase pathway during hypoxia and ischemia (Li *et al.*, 2004). This nitric oxide pathway is also involved in the contraction stimulation pathway as a method of increasing glucose transport during exercise (Roberts *et al.*, 1997).

Other than insulin, several other hormones can also regulate GLUT-mediated glucose uptake at an expression level, rather than influencing the translocation. Thyroid hormones regulate both by promoting GLUT 4 expression while leading to decreased GLUT 1 levels in heart and brown adipose tissue in neonates (Castello *et al.*, 1994). In the liver thyroid hormone has been shown to regulate the expression of GLUT 2 in order to control hepatic glucose metabolism (Weinstein *et al.*, 1994). The fructose transporter GLUT 5 is also regulated by thyroid hormone in the presence of glucose (Matosin-Matekalo *et al.*, 1999). GLUT 1 and GLUT 3 show regulation by glucocorticoids in the placenta (Hahn *et al.*, 1999). GLUT 1 glucocorticoid related expression is also apparent in early growth retarded, hypertensive, hyperinsulinaemic male rats in conjunction with activation of Akt (Langdown *et al.*, 2001). Other hormones, such as retinoic acid (GLUT 4) (Sleeman *et al.*, 1995) human chorionic gonadotropin (hCG) (GLUT 8), have been implicated in GLUT regulation (Chen *et al.*, 2003).

5.3.4 SSAO and glucose transporters

SSAO was identified, through the oxidation of methylamine, in both white and brown adipose tissue homogenates from rats and in mature adipocytes isolated from these tissues (Conforti *et al.*, 1993). This led to the possibility of a role in adipose tissue function. Furthermore, oxidation of both benzylamine and histamine, which was eliminated by specific SSAO inhibitors, confirmed the presence of SSAO (Ramondi *et al.*,1997). Substantial levels of SSAO were localized to the membrane of adipocytes but immunoisolation studies also indicated the presence of SSAO within GLUT 4 containing vesicles.

An increase in glucose uptake was detected in the presence of an amine substrate (benzylamine) (Enrique-Tarancon *et al.*,1998). The physiological substrate tyramine, which is also a MAO substrate, gave a weak stimulation of glucose transport. However,

this stimulation was markedly increased in the presence of vanadate. The main effector in this system appears to be the H_2O_2, produced as a product of the oxidative deamination of the amine substrate by the amine oxidases, since the stimulatory effect can be readily blocked by the addition of catalase to the assay system (Marti *et al.*, 1998). The increase of glucose uptake is due to an increase in the translocation of the GLUT 4 transporter from the GLUT-4 containing vesicles to the cell membrane. Stimulation of the translocation via amine substrates is mediated through the PI3K pathway after tyrosine phosphorylation of the insulin receptors IRS-1 and IRS-3 (Enrique-Tarancon *et al.*, 2000). Further to the insulin-like stimulation of glucose uptake, the addition of amine substrates causes the inhibition of lipolysis. This inhibition is blocked by anti-oxidants, also suggesting a role for the H_2O_2 produced from the amine oxidase reaction (Morin *et al.*, 2001). Peroxyvanadate is formed in the presence of vanadate and H_2O_2 and this can mimic insulin effects. It is this compound that is believed to mediate the stimulation of the insulin-like effects seen in the presence of amine oxidases substrates and vanadate. However, another peroxovanadium compound, bis-peroxovanadium-1-10-phenanthrolene, stimulated both the insulin and contraction pathways alone (Nolte *et al.*, 2003).

H_2O_2 is also responsible for adipocyte maturation in 3T3-L1cells, as methylamine induced maturation was prevented by catalase. This effect appears exclusive to SSAO, since as MAO inhibitors had no apparent effect (Mercier *et al.*, 2001). The insulin- like effects of amine substrates is not limited to adipocytes and has been reported in other insulin-sensitive tissues and without the need for vanadate (Morin *et al.*, 2002). Studies where insulin resistant animals show reduced glucose uptake in response to insulin treatment, which was overcome by treatment with benzylamine and vanadate, suggesting a possible means of treating this form of diabetes (Abella *et al.*, 2003). However, the improvement appears only to in insulin-depleted models and diabetic animals receiving tyramine for three weeks showed no improvement from hyperglycemia, hypoinsulinemia or glucosuria but only in glucose tolerance (Visentin *et al.* 2003). Recent animal studies have shown the insulin-like effects of SSAO in the presence of a substrate to be a physiological reality in rabbits and diabetic mice without the necessity of added vanadate (Iglesias-Osma *et al.*, 2004). Present studies have now begun to focus on amine oxidase

functions in human adipose tissue and in non-obese subjects where adipocytes express a membrane bound SSAO. This SSAO will oxidize amine substrates and can exert short-term insulin like effects on glucose and lipid metabolism and hopefully these studies will expand our understanding of this function of SSAO under varying conditions (Carpene *et al.*, 2003).

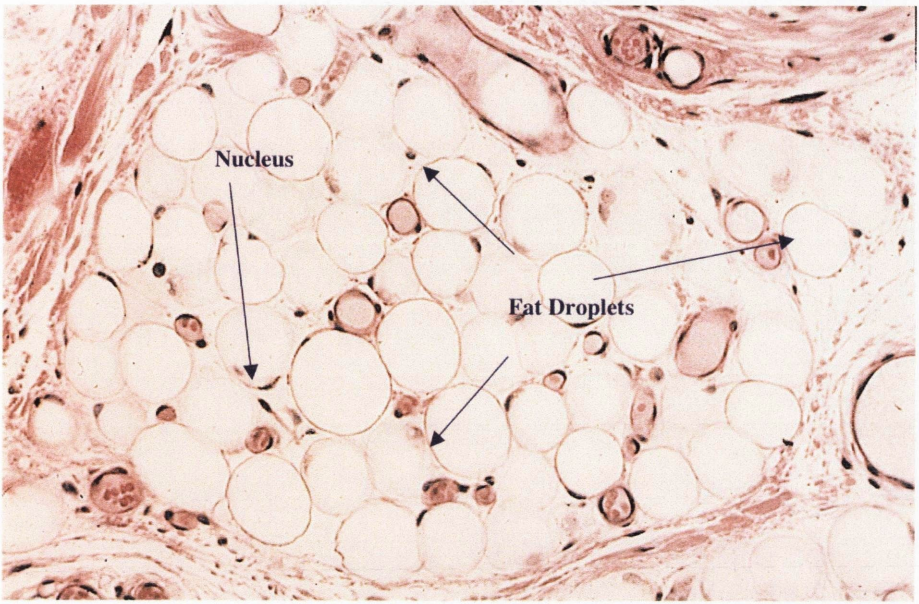
5.4Adipocytes

Adipocytes are cells found in deposits of adipose tissue, which is a loose connective tissue primarily used by the body as an energy store. Adipose tissue is located beneath the skin and around internal organs where it acts as an insulator and offers protection (Goodpaster 2002). Two types of adipose tissue exist, white adipose tissue (WAT) and brown adipose tissue (BAT), containing white adipocytes and brown adipocytes respectively.

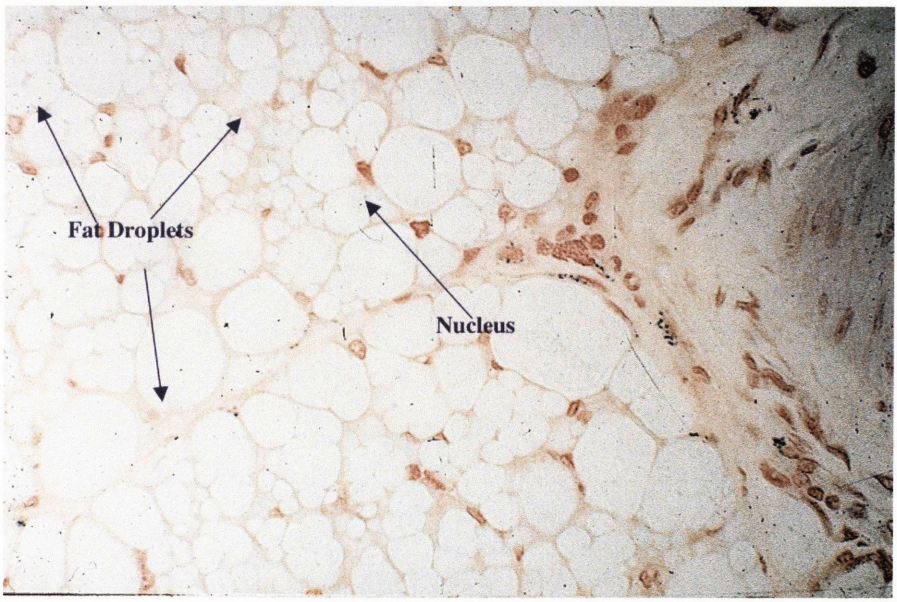
In white adipose tissue, the adipocyte cytoplasm is dominated by a single large fat vacuole. The cell nucleus and other organelles are pushed by the vacuole to the periphery. The fat is stored in a semi-liquid state and is composed of triglycerides. Brown adipose tissue is more widely represented in fetuses. The main function of brown adipose tissue is the production of heat via non-shivering thermogenesis. This is achieved through the uncoupling of the electron transport chain from the production of ATP by uncoupling protein 1 (UCP1) allowing the energy produced by the electron transport chain to be released as heat (Nedergaard *et al.*, 2001). In contrast to the single fat droplet which exists in white adipocytes, brown adipocytes contain several smaller vacuoles with lipid droplets scattered throughout. The nucleus in brown adipocytes is randomly located but is not pushed to the periphery of the cell. The colour of brown adipocytes is due to the amount of iron-containing pigments within the cell. See Fig. 5.2 for representative slides showing white and brown adipocytes.

Fig 5.2. Representative slides showing white adipocytes (A) and brown adipocytes (B). Magnification X 400.

A



B

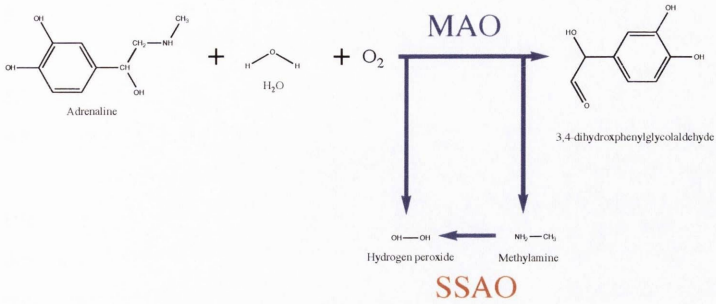


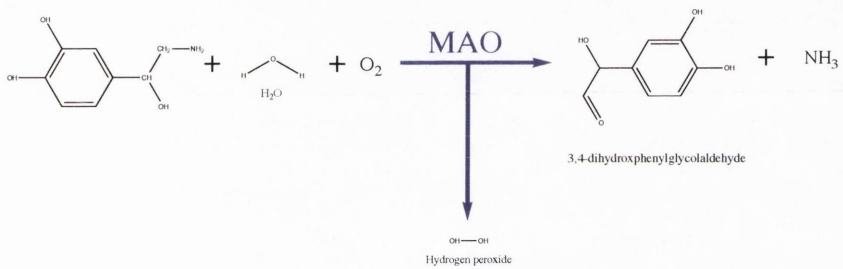
5.5 Aims

Adrenaline and noradrenaline (see Fig 5.4 below for the structures) have both been implicated in stimulating glucose transport in white and brown adipose tissue (Faintreine & Geloen 1998, Chernogubova *et al.*, 2004). Stimulation of glucose uptake is normally mediated through the adrenergic receptors and eventual activation of the PI3K pathway. Both adrenaline and noradrenaline are amines and substrates for both MAO isoenzymes (Tipton *et al* 2004). Although adrenaline and noradrenaline are not substrates for SSAO, oxidation by MAO will give rise to methylamine, which is a substrate for SSAO, see Fig 5.4. As discussed previously, the oxidation of amines by SSAO and MAO in adipose and other tissue causes an increase in glucose uptake due to the translocation of GLUT 4. This translocation is believed to be due to the production of H_2O_2, which is a known intracellular signalling molecule (Rhee 1999).

As both physiological and non-physiological amines cause the increase of glucose transport, this work was designed to examine whether the physiological amines adrenaline and noradrenaline can, either in conjunction with or via a distinct mechanism from the adrenergic receptor mediated pathway, regulate the translocation of GLUT 4 to enable an increase in glucose uptake.

Fig. 5.4. The chemical structures of adrenaline and noradrenaline and the structures of the products by their oxidation by Monoamine Oxidase (MAO) and Semicarbazide Sensitive Amine Oxidase (SSAO).


Adrenaline
H_2O
O_2
MAO
3,4-dihydroxphenylglycolaldehyde
Hydrogen peroxide
Methylamine
SSAO


H_2O
O_2
MAO
NH_3
3,4-dihydroxphenylglycolaldehyde
Hydrogen peroxide

5.7 Results

5.7.1 Determination of total amine oxidase activity in adipocytes isolated from rat white adipose tissue.

Utilising common substrates and selective inhibitors of each enzyme it was possible to determine the amine oxidase composition of the adipocytes. SSAO was found to comprise of about 75% of the activity with the reminder consisting of ~18% MAO A and ~7% MAO B (Fig. 5.7.1).

5.7.2 Effects of amine oxidase inhibitors on basal glucose uptake in adipocytes isolated from rat white adipose tissue

Adipocytes were isolated as described in Section 2.8. Basal glucose uptake was determined in the presence of each of the amine oxidase inhibitors used in this study. After 30 min incubation with the inhibitors, clorgyline and deprenyl (1 μM) showed slight, but not statistically significant, inhibition of glucose uptake. Although semicarbazide (1 mM) resulted in a greater inhibition, this was not significantly different from that seen with clorgyline and deprenyl when compared to the control($p = 0.13$; Fig 5.7.2).

5.7.3 Comparison of glucose uptake stimulation by insulin, (-)-adrenaline, (±)-adrenaline and noradrenaline.

Adipocytes were isolated as described in Section 2.8. All stimulants resulted in a significant increase in glucose uptake, over the basal level. There were no significant differences in the uptake by the different stimulants. Insulin increased glucose uptake by 44 ± 0.7%, (-)-adrenaline increased uptake by 55 ± 14%, (±)-adrenaline increased uptake by 49 ± 12% and noradrenaline increased uptake by 41 ± 14% (see Fig. 5.7.3).

5.7.4 Effects of monoamine oxidase inhibitors, clorgyline and deprenyl, on (±)-adrenaline, (-)-adrenaline and noradrenaline mediated glucose uptake in adipocytes isolated from rat white adipose tissue

Glucose uptake mediated by the addition of adrenaline and noradrenaline to isolated adipocytes, determined as described in Section 2.8, was reduced in the presence of monoamine oxidase inhibitors. Uptake levels are expressed as a percentage increase in the basal uptake level. (-)-Adrenaline alone stimulated glucose uptake by 71 ± 3% and this was reduced to a stimulation of 47 ± 10% of basal uptake in the presence of the inhibitors with a significance of $p< 0.0059$ (see Fig. 5.7.4). (±)-Adrenaline alone stimulated glucose uptake by 50 ± 3%, however in the presence of clorgyline (1 μM) this was significantly reduced to 18 ± 2.5%, $p < 0.001$ (see Fig. 5.7.5). Noradrenaline alone stimulated glucose uptake by 57 ± 6%, however uptake was essentially abolished in the presence of the inhibitors with only a 1 ±0.25% increase over basal levels (see Fig. 5.7.6).

5.7.5 Effects of the semicarbazide sensitive amine oxidase inhibitor, semicarbazide, on glucose uptake stimulated by the presence of (±)-adrenaline, (-)-adrenaline and noradrenaline in adipocytes isolated from rat white adipose tissue.

Adipocytes were isolated as described in Section 2.8. Glucose uptake stimulated by the addition of adrenaline was reduced in the presence of the semicarbazide-sensitive amine oxidase inhibitor, semicarbazide, however the uptake stimulated by the presence of noradrenaline was unchanged. Uptake levels are expressed as a percentage increase in the basal uptake level. (-)-Adrenaline alone stimulated glucose uptake by 36 ± 3.5% with a reduction to 7 ± 0.5% of basal uptake in the presence of the inhibitor with a significance of $p < 0.001$ (see Fig. 5.7.7). (±)-Adrenaline alone stimulated glucose uptake by 85 ± 9%, however in the presence of semicarbazide this was significantly reduced to 18 ± 2.5% ($p < 0.001$; see Fig. 5.7.8). Noradrenaline alone stimulated glucose uptake by 36.5 ± 6.5%, however in the presence of the inhibitor no change in glucose uptake was observed with uptake remaining at 36 ± 5% over basal levels (see Fig. 5.7.9).

5.7.6 Effects of the combination of the amine oxidase inhibitors clorgyline, deprenyl and semicarbazide on (±)-adrenaline, (-)-adrenaline and noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue.

Glucose uptake stimulated by the addition of adrenaline and noradrenaline to isolated adipocytes was reduced in the presence of amine oxidase inhibitor mixture (-)-Adrenaline alone stimulated glucose uptake by 56 ± 11% with a reduction to 5 ± 0.5% of basal uptake in the presence of the inhibitor with a significance of $p < 0.001$ (see Fig. 5.7.10). (±)-Adrenaline alone stimulated glucose uptake by 33 ± 6%, however in the presence of semicarbazide, clorgyline and deprenyl this was significantly reduced to 3.25 ± 0.2% of basal uptake ($p < 0.001$; see Fig. 5.7.11). Noradrenaline alone stimulated glucose uptake by 72 ± 11.5%, however uptake was reduced in the presence of the inhibitors to 5.3 ±0.4% increase over basal level, $p < 0.001$, compared to the value without inhibitors (see Fig. 5.7.12).

5.7.7 Effects of catalase on (±)-adrenaline, (-)-adrenaline and noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue.

Catalase (15 units) reduced the glucose uptake stimulated by adrenaline and noradrenaline to isolated adipocytes Catalase itself had no effect on basal glucose uptake at the units of activity used. (-)-Adrenaline alone stimulated glucose uptake by 49 ± 5.5% and this was reduced to 9 ± 1% of basal uptake in the presence of catalase, with a significance of $p < 0.001$(see Fig. 5.7.13). (±)-Adrenaline alone stimulated glucose uptake by 65 ± 20% and this was reduced to 1.5 ± 0.1% of basal uptake in the presence of catalase ($p < 0.001$; see Fig. 5.7.14). Noradrenaline alone stimulated glucose uptake by 53 ± 7%, however uptake was reduced in the presence catalase to a 2± 0.1% increase over basal level ($p < 0.001$; see Fig. 5.7.15).

5.7.8. Effects of DSA (Diazotised sulphanilic acid) 280 μM on (±)-adrenaline, (-)-adrenaline and noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue.

DSA had no significant effect on (±)-adrenaline or (-)-adrenaline stimulated glucose uptake. However a slight increase was observed in the presence of DSA.

5.7.9 Effects of vanadate on (±)-adrenaline, (-)-adrenaline and noradrenaline noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue.

Vanadate in the presence of adrenaline caused an increase in glucose uptake in comparison to adrenaline alone. No significant difference was observed in the action of noradrenaline. However vanadate alone showed a significant increase in basal glucose uptake levels. (-)-Adrenaline alone stimulated glucose uptake by 27 ± 1.5% with an increase to 57 ± 4.5% in the presence of vanadate, however vanadate alone in this system showed a 35 ± 3.5% increase in glucose uptake (see Fig 5.7.16). (±)-Adrenaline alone stimulated glucose uptake 43.5 ± 2% and in the presence of vanadate glucose uptake increase to 85 ± 6%, however vanadate alone resulted in a 105 ± 13% increase (see Fig. 5.7.17Noradrenaline alone caused an increase of 71 ± 6% in uptake. However, vanadate made no significant difference to the uptake of glucose, although vanadate alone still resulted in an increase of 41 ± 4% in glucose uptake (see Fig 5.7.18). The presence of catalase (15 units) did, however, reduce vanadate mediated glucose uptake, 86 ± 4% increase on basal levels as compared to 40 ± 2% increase on basal levels in the presence of catalase (see Fig. 5.7.19).

5.7.10 Effects of an adrenaline uptake inhibitor on (-)-adrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue.

The adrenergic uptake inhibitor maprotiline reduced glucose uptake stimulated by adrenaline. (-)-Adrenaline alone stimulated glucose uptake by 28 ± 14% and in the

presence of metaprotiline glucose uptake was significantly reduced to below basal level (–2.3 ± 1.9%, $p < 0.045$; see Fig. 5.7.20).

5.7.11 Effects of α-and β-adrenergic antagonists on (±)-adrenaline and noradrenaline stimulated glucose uptake in adipocytes isolated from rat white adipose tissue.

No significant effects on glucose uptake stimulated by (±)-adrenaline or noradrenaline was observed by the addition of α- and β-adrenergic antagonists (see Fig. 5.7.21).

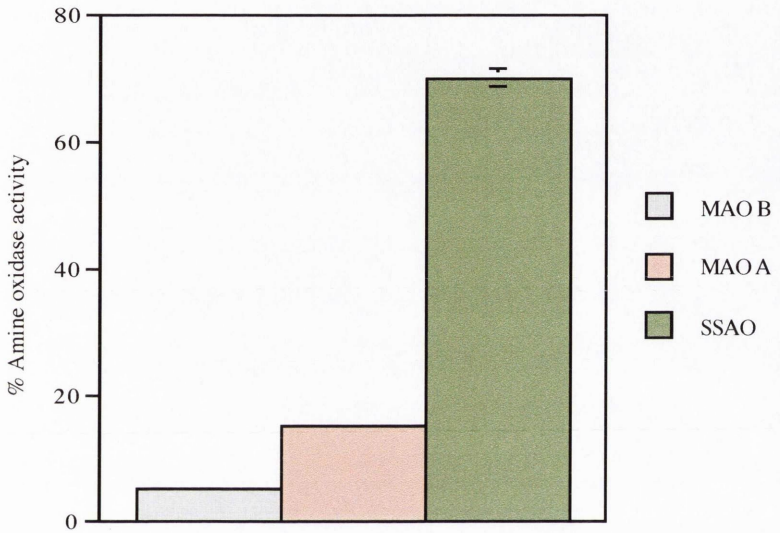


Fig. 5.7.1. Amine oxidase activities in adipocytes isolated from male wistar rat epidimal pads.

Amine oxidase assays were determined in adipocyte suspensions (10µl) using ^{14}C-Tyramine as a common substrate for SSAO, MAO A and MAO B. Semicarbazide (1mM), clorgyline(1µM) and deprenyl(1µM) were used as specific inhibitors against each enzyme respectively. All samples were preincubated with inhibitor for 30 min at 37°C in a shaking water bath. The reaction was initiated by the addition of substrate and terminated by acidification after 15 min (Tipton *et al.*, 2000). Total amine oxidase activity was 0.42 nmol/min/mg protein. The results shown are mean values ± S.E.M. of at least 9 replicates.

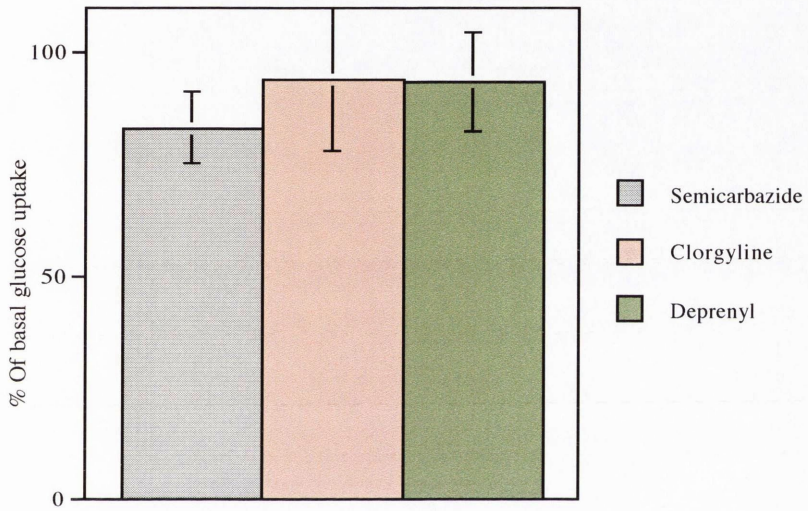


Fig. 5.7.2. The effects of amine oxidase inhibitors on basal glucose uptake levels by adipocytes from from male wistar rat epidimal pads.

Adipocytes (100µl) were incubated with semicarbazide (1mM), clorgyline (1µM) and deprenyl (1µM) for 30 min at 37°C. Glucose uptake levels were determined by the incubation of the adipocytes with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with any ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 4.47 ± 0.4 pmol/min/ml adipocyte suspension. The results shown are mean values ± S.E.M. of at least 9 replicates.

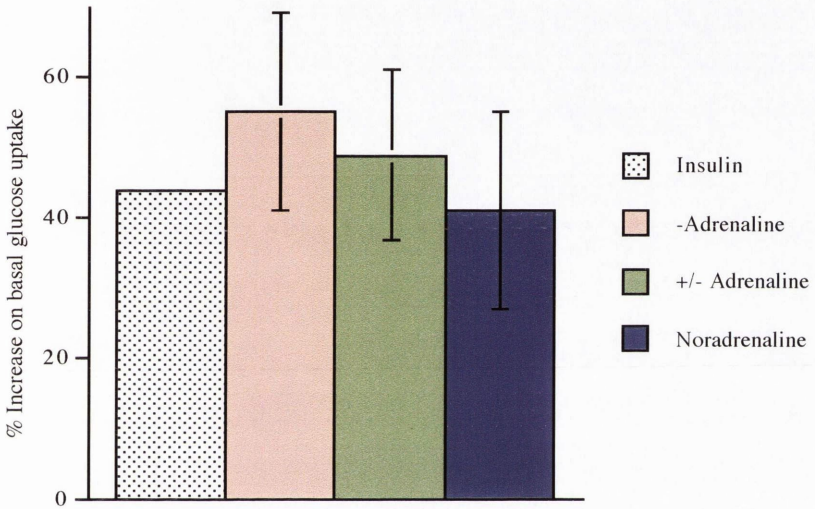


Fig. 5.7.3. Comparison of glucose uptake stimulation by insulin (0.0001mg/ml), (-)-adrenaline (10μM), (±)-adrenaline (10μM) and noradrenaline (10μM).

Adipocytes were incubated with each of stimulators for 45 min at 37°C. Glucose uptake levels were determined by the incubation of the adipocytes with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration and ^{3}H-2-deoxyglucose retained within the adipocytes was measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 2.57 ± 0.45 pmol/min/ml adipocyte suspension.The results shown are mean values ± S.E.M. of at least 9 replicates.

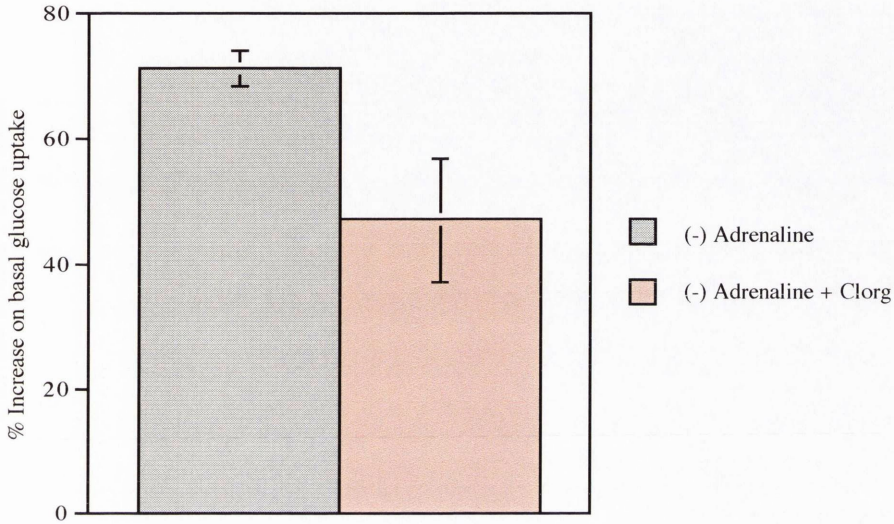


Fig. 5.7.4. The effect of the MAO-A inhibitor clorgyline on (-)-adrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with clorgyline (1μM) for 30 min at 37°C. Stimulation of the adipocytes with (-)-adrenaline (10 μM) was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 4.77 ± 0.34 pmol/min/ml adipocyte suspension. The results shown are mean values ± S.E.M. of at least 9 replicates.

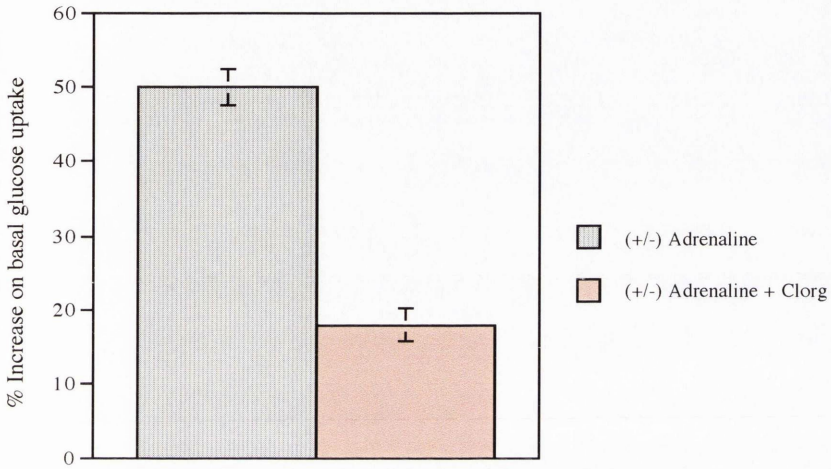


Fig 5.7.5. The effects of the MAO-A inhibitor clorgyline on (±)-adrenaline (10μM) stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100μl) were incubated with clorgyline (Clorg;1μM) for 30 min at 37°C. Stimulation of the adipocytes with (±)adrenaline was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with any ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 3.9 ± 0.8 pmol/min/ml adipocyte suspension. The results shown are mean values ± S.E.M. of at least 9 replicates.

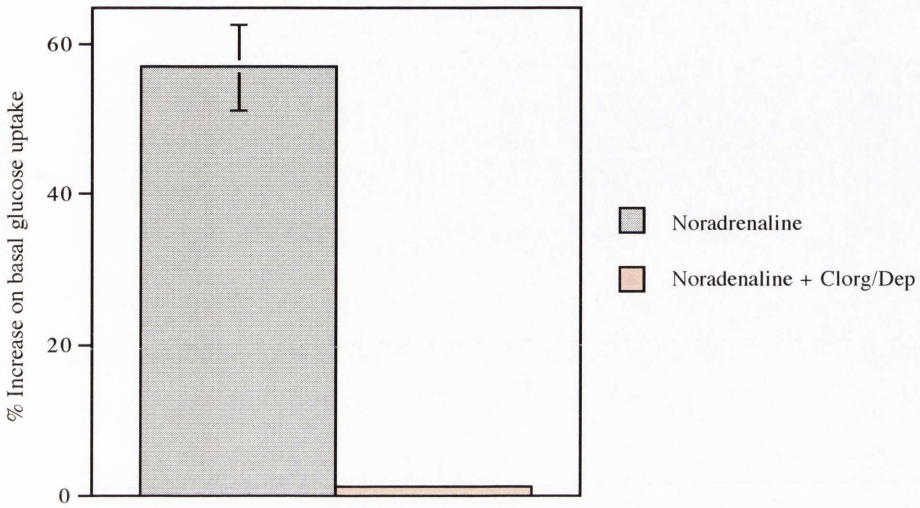


Fig. 5.7.6. The effects of the MAO-A inhibitor, clorgyline and the MAO-B inhibitor, deprenyl on noradrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100µl) were incubated with 1 μM clorgyline (Clorg) plus 1 μM deprenyl (Dep) for 30 min at 37°C. Stimulation of the adipocytes with 10µM noradrenaline was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with any ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 5.65 ± 0.95 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

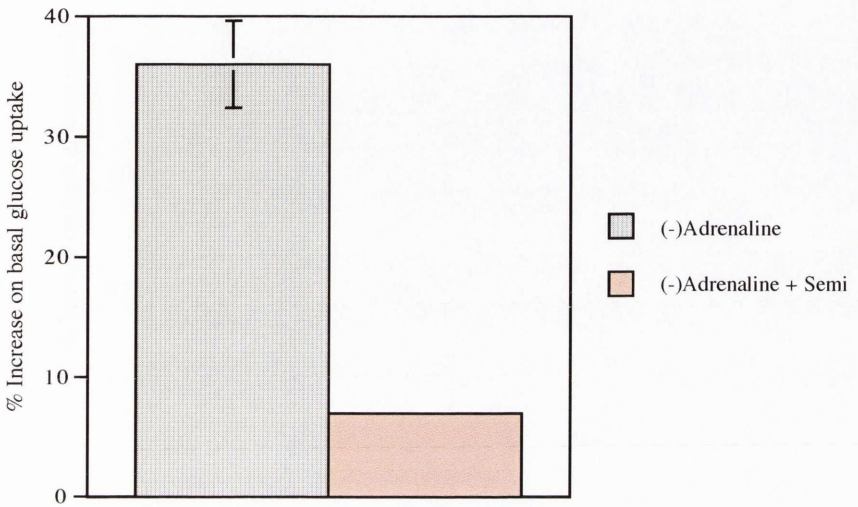


Fig. 5.7.7. The effects of the SSAO inhibitor, semicarbazide, on (-)-adrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100µl) were incubated with 1 mM semicarbazide (Semi) for 30 min at 37°C. Stimulation of the adipocytes with (-)-adrenaline (10 µM) was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 4.50 ± 0.5 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

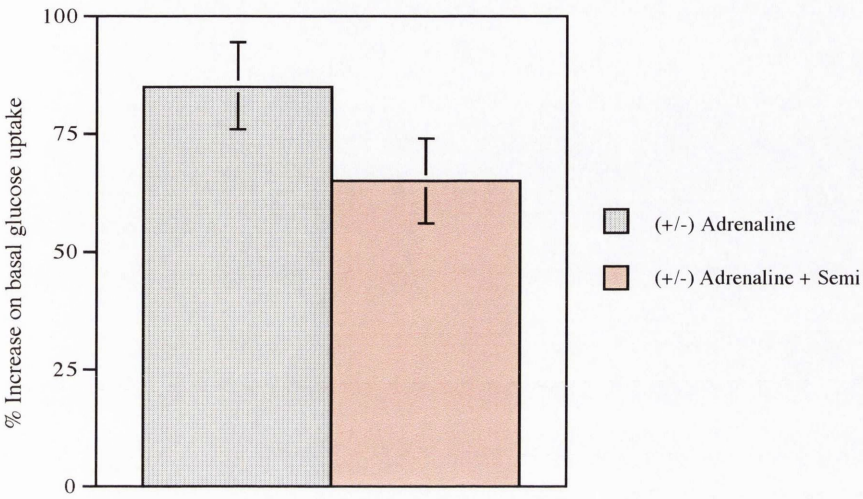


Fig. 5.7.8. The effects of the SSAO inhibitor, semicarbazide, on (±)-**adrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.**.

Adipocytes (100 μl) were incubated with 1 mM semicarbazide (Semi) for 30 min at 37°C. Stimulation of the adipocytes with (±)-adrenaline was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). The results shown are mean values ± S.E.M. of at least 9 replicates.

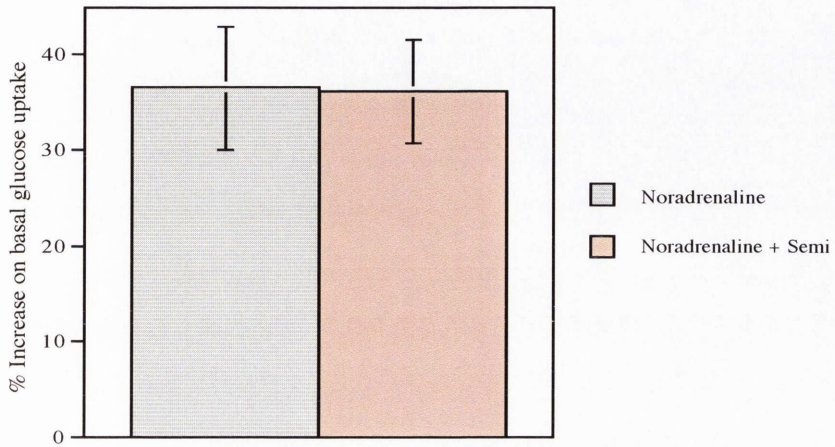


Fig. 5.7.9. The effects of the SSAO inhibitor, semicarbazide, on nordrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with with 1 mM semicarbazide (Semi) for 30 min at 37°C. Stimulation of the adipocytes with nordrenaline was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 2.63 ± 0.3 pmol/min/ml adipocyte suspension. The results shown are are mean values ± S.E.M. of at least 9 replicates.

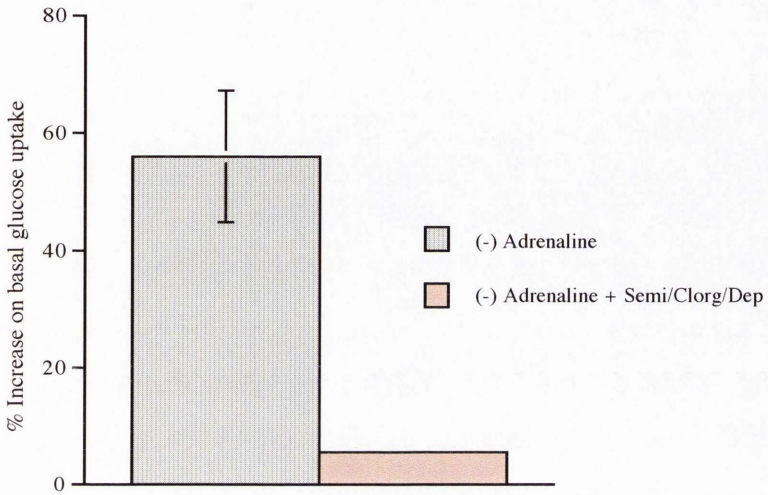


Fig. 5.7.10. The effects of amine oxidase inhibition on the (-)-adrenaline stimulated glucose uptake by adipocytes from from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with1 mM semicarbazide, 1 μM clorgyline plus 1 μM deprenyl (Semi/Clorg/Dep) for 30 min at 37°C. Stimulation of the adipocytes with (-)-adrenaline (10 μM) was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 1.62 ± 0.02 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

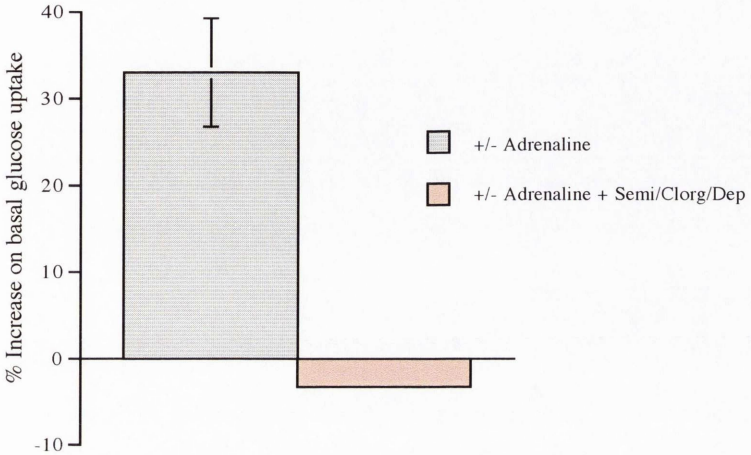


Fig. 5.7.11. The effects of amine oxidase inhibition on (±)-adrenaline stimulated glucose uptake by adipocytes from from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with 1 mM semicarbazide, 1 μM clorgyline plus 1 μM deprenyl (Semi/Clorg/Dep) for 30 min at 37°C. Stimulation of the adipocytes with (±)-adrenaline was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 2.35 ± 0.3 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

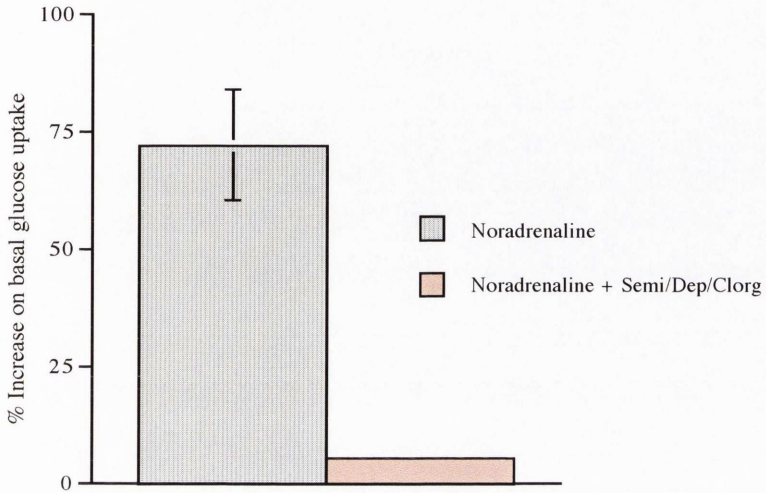


Fig. 5.7.12. The effects of amine oxidase inhibition on noradrenaline stimulated glucose uptake by adipocytes from from male wistar rat epidimal pads..

Adipocytes (100 μl) were incubated with1 mM semicarbazide, 1 μM clorgyline plus 1 μM deprenyl (Semi/Clorg/Dep) for 30 min at 37°C. Stimulation of the adipocytes with noradrenaline (10 μM) was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 5.1 ± 1.1 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

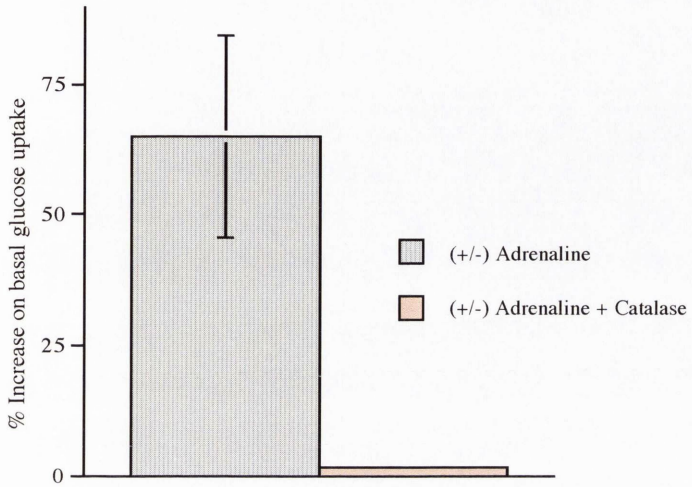


Fig. 5.7.13. The effect of catalase on (±)-adrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with catalase (15 units) for 30 min at 37°C, stimulation of the adipocytes with (±)-adrenaline (10 μM) was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 1.83 ± 0.2 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

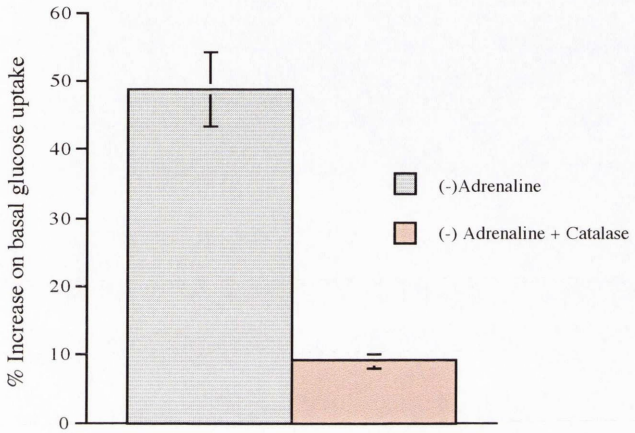


Fig. 5.7.14. The effect of catalase on (-)-adrenaline stimulated uptake adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with catalase (15 units) for 30 min at 37°C, stimulation of the adipocytes with (-)-adrenaline (10 μM) was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 2.53 ± 0.17 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

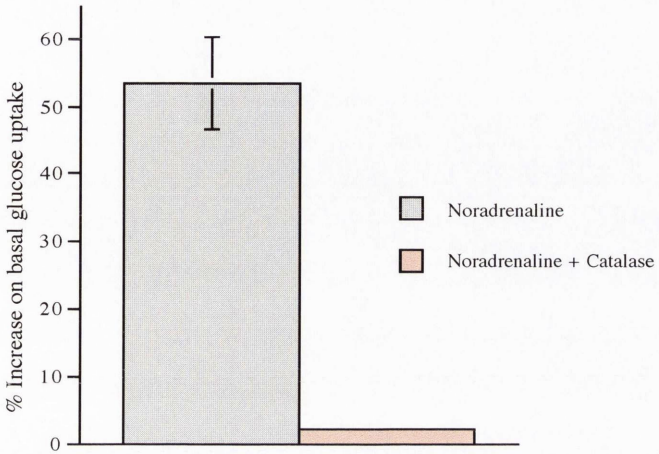


Fig. 5.7.15. The effect of catalase on noradrenaline stimulated uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with catalase (15 units) for 30 min at 37°C, stimulation of the adipocytes with (-)-adrenaline (10 μM) was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 2.93 ± 0.24 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

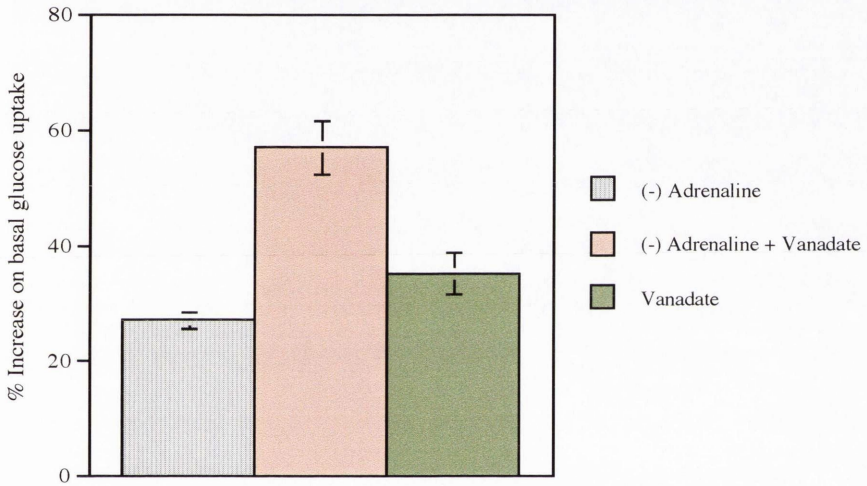


Fig. 5.7.16. The effect of vanadate on (-)-adrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads..

Adipocytes (100 μl) were stimulated with (-)-adrenaline (10 μM) in the presence of vanadate (100 μM) for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 5.83 ± 0.6 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

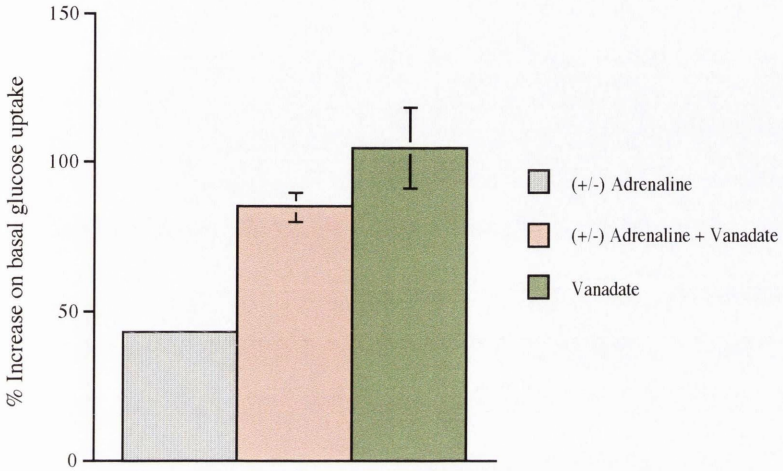


Fig. 5.7.17. The effect of vanadate on (±)-adrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were stimulated with (±)-adrenaline (10 μM) in the presence of vanadate (100 μM) for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 6 ± 0.51 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

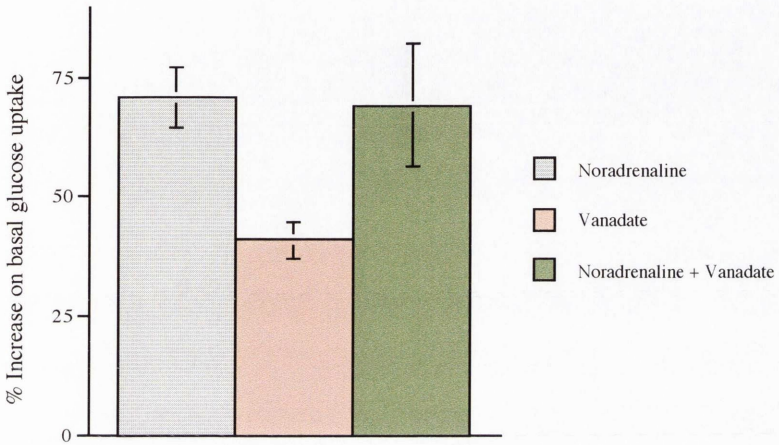


Fig. 5.7.18. The effect of vanadate on noradrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads..

Adipocytes (100 μl) were stimulated with noradrenaline in the presence of vanadate (100 μM) for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 3.73 ± 1.13 pmol/min/ml adipocyte suspension. The results shown are mean values ± S.E.M. of at least 9 replicates.

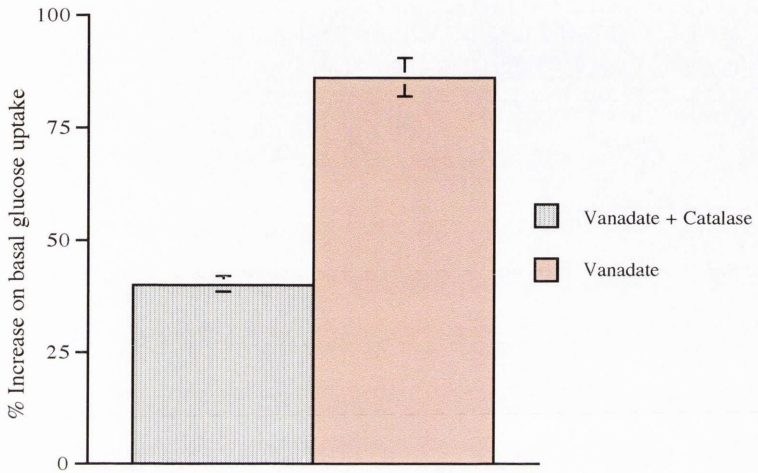


Fig. 5.7.19. The effect catalase plus vanadate on basal glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with catalase (15 units) and vanadate (100 μM) for 20 min at 37°C. Glucose uptake levels were determined by the incubation of the adipocytes with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 1.6 ± 0.17 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

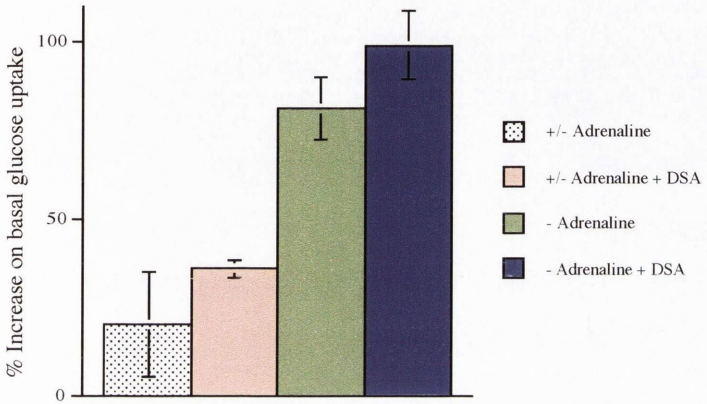


Fig. 5.7.19. The effect of DSA on (±)-adrenaline and (-)-adrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with DSA (280 μM) before addition of (±)-adrenaline (10 μM) and (-)-adrenaline (10 μM)for 15 min at 37°C. Glucose uptake levels were determined by the incubation of the adipocytes with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 5.15 ± 0.15 pmol/min/ml adipocyte suspension The results shown are mean values ± S.E.M. of at least 9 replicates.

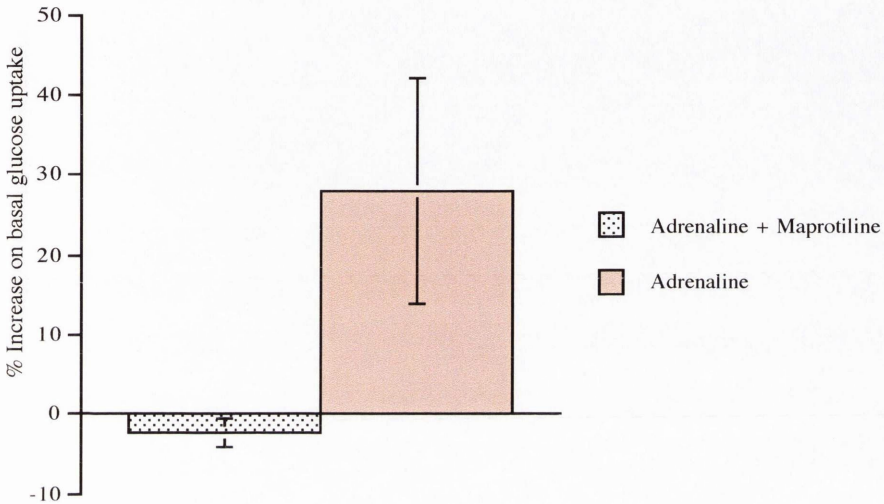


Fig. 5.7.20. The effect of the uptake inhibitor, maprotiline (1μM) on (-)-adrenaline stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes were incubated with maprotiline for 30 min at 37°C. Stimulation of the adipocytes with (-)-adrenaline was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 3.73 ± 1.67 pmol/min/ml adipocyte suspension. The results shown are mean values ± S.E.M. of at least 9 replicates.

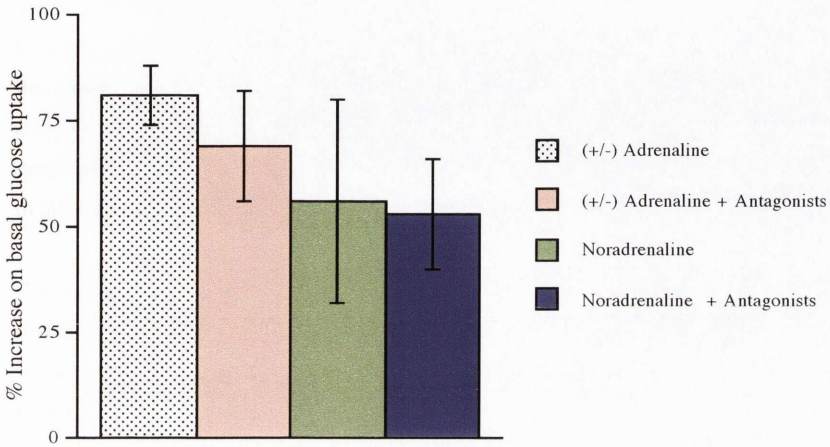


Fig. 5.7.21. The effect of the α-adrenergic antagonist, phentolamine and the β-adrenergic antagonist, propranolol on (±)-adrenaline and noradrenaline (10μM) stimulated glucose uptake by adipocytes from male wistar rat epidimal pads.

Adipocytes (100 μl) were incubated with phentolamine (1 μM) and propranolol (1 μM) for 30 min at 37°C. Stimulation of the adipocytes with (-)adrenaline (10 μM) was carried out for 45 min followed by glucose uptake with ^{3}H-2-deoxyglucose for a further 10 min. Glucose uptake was terminated by filtration with any ^{3}H-2-deoxyglucose retained within the adipocytes measured by scintillation counting (Morin *et al.*, 2001). Basal uptake was 4.63 ± 0.58 pmol/min/ml adipocyte suspension. The results shown are ± S.E.M. of at least 9 replicates.

5.8 Discussion

There were variations in the basal levels and the levels of stimulation by the amines between different adipocyte preparations. These may have been a result of differences in the metabolic states of each animal used. Practical limitations restricted the number of assays that could be performed on each adipocyte preparation. Although this restricted quantitative inter-preparation comparisons, the stimulatory effects were seen consistently in all samples.

Previous studies have used adipocytes (both isolated and cultured) to study the effects of SSAO substrates on mediation of glucose uptake. The discovery that intracellular SSAO was associated with GLUT4 containing vesicles led to the realisation that a possible role may exist for SSAO regulation of glucose uptake due to related enzyme activity (Enrique-Tarancón *et al.*, 1998). In most studies the substrates used have been benzylamine and tyramine. Benzylamine, which is also a substrate for MAO-B, is a non-physiological compound and tyramine is a substrate for MAO A and MAO B as well as SSAO. Possible physiological substrates for this process are methylamine and aminoacetone. However, in the circulating blood these are likely targets for oxidation by plasma and membrane-bound SSAO. One realistic physiological substrate is adrenaline. Although not metabolised by SSAO (Boomsma *et al.*, 1993), it does undergo oxidative deamination catalysed by MAO. Once oxidised by MAO, one of the products is methylamine, which can then be oxidatively deaminated by SSAO (Fig. 5.8.1).

Adrenaline, a member of biogenic amine family was first isolated by two independent groups (Takamine, 1901; Aldrich, 1901). Adrenaline functions both as a hormone where it stimulates cellular glucose metabolism and as a neurotransmitter. It has been shown to stimulate glucose uptake by isolated adipocytes through mechanisms that involve both α- and β-adrenergic receptors (Colivicchi *et al.*, 2004). This study set out to determine whether another possible role for adrenaline might be the stimulation of GLUT4 mobilisation through H_2O_2 formation

Determination of the amine oxidase proportions of adipocytes showed SSAO activity to predominate along with smaller amounts of both MAO A and B. Previous studies have shown that the product of amine oxidation, hydrogen peroxide, is the intermediated used to mediate the translocation of GLUT-4 containing vesicles to the cell surface, thereby increasing glucose uptake, and that both MAO and SSAO can contribute to this (see e.g., Enrique-Tarancón *et al.*, 1998b; Morin *et al.*, 2002).

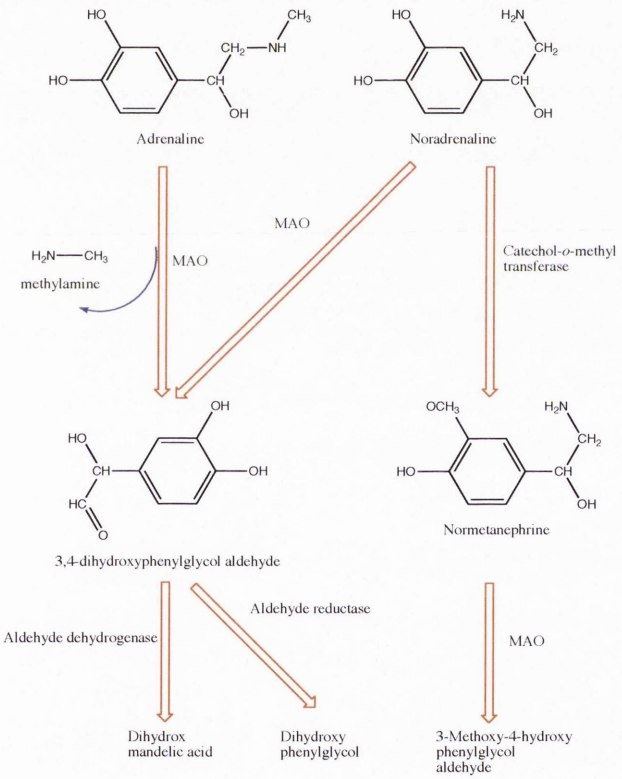


Fig. 5.8.1. Outline of the initial stages of the normal adrenaline and noradrenaline catabolism.

Addition of two forms of adrenaline, (-) and (±) along with noradrenaline induced an increase in glucose uptake. Normal biosynthesis of adrenaline results in the (-) form of the molecule (see Fig. 5.8.2). (±) Adrenaline was used to determine if any stereospecific effective on glucose uptake was observed, since the (+) form was not commercially available. At the concentrations used (10 μM) there appeared to be little difference in their stimulatory effects, which were similar to those found with insulin (0.0001 mg/ml).

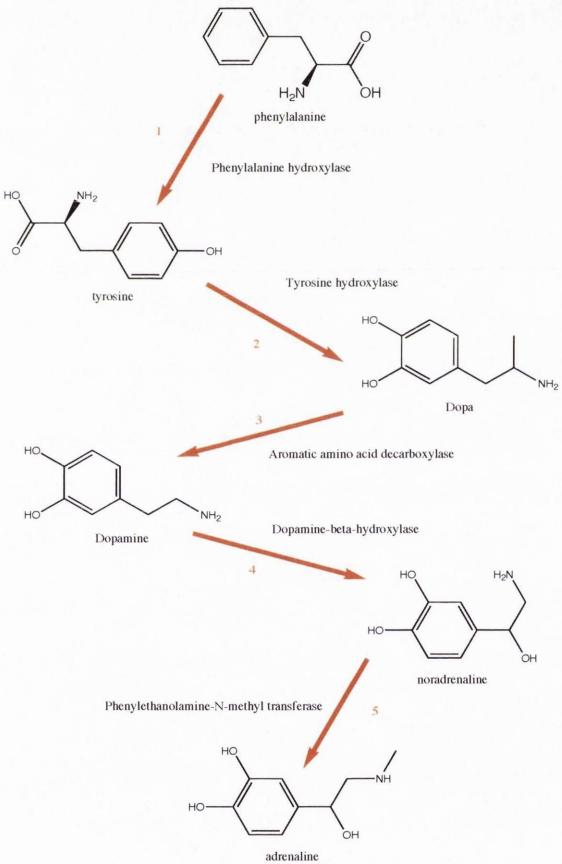


Fig. 5.8.2. Biosynthetic pathway of adrenaline.

In the presence of MAO inhibitors this stimulated uptake was reduced, suggesting the hydrogen peroxide produced by the MAO-catalysed oxidation of noradrenaline and adrenaline to be involved. However, the stimulation by noradrenaline was unaffected by the presence of an SSAO inhibitor. This might be expected, since methylamine is not a product of the MAO-catalysed oxidation of noradrenaline. In contrast, the stimulation by adrenaline was reduced by SSAO inhibition. This suggests that hydrogen peroxide produced by the oxidation of methylamine is also involved. Further evidence suggests that the oxidation responsible for the hydrogen peroxide is limited to that within the cell. DSA a potent SSAO inhibitor, which is unable to enter the cell (Holt & Callingham, 1994), would block any hydrogen peroxide derived from SSAO outside the cell. No effect on glucose uptake was observed in the presence of DSA apart from a slight (but not significant) increase which would be explained by a intracellular increase in methylamine availability given the inhibition of extracellular SSAO. See Fig. 5.8.3 for an outline of the proposed mechanism.

The presence of catalase reduced the stimulated glucose uptake under all conditions used here, confirming the involvement of hydrogen peroxide. Many studies have included vanadate and it is believed that peroxyvandate, formed by its reaction with hydrogen peroxide is a more effective signalling molecule in the GLUT-4 translocation. Vanadate alone has been shown to exert insulin-like effects (for review, see Goldwaser *et al,*, 2000) and, in the present studies, it was found to be capable of stimulating glucose uptake on its own. This might, in part, result from the formation of peroxyvanadate with endogenous hydrogen peroxide, perhaps produced through the action of lipolytic hormones on free fatty acids (Muchmore *et al.,* 1982), in the system used. However, the addition of catalase only resulted in a 50% reduction of vanadate-stimulated glucose uptake. Nevertheless there was no indication of enhancement of the adrenergic stimulation in the presence of vanadate. The action of vanadate alone shows caution must be taken when analysing such data as previous studies have shown (Goldwaser *et al.*, 2000)

Adrenaline and noradrenaline will of course stimulate glucose uptake through adrenergic receptors (Nonogaki, 2000), however in isolated adipocytes desensitisation of the receptors was common, possibly as a result to the isolation procedure. This is evident

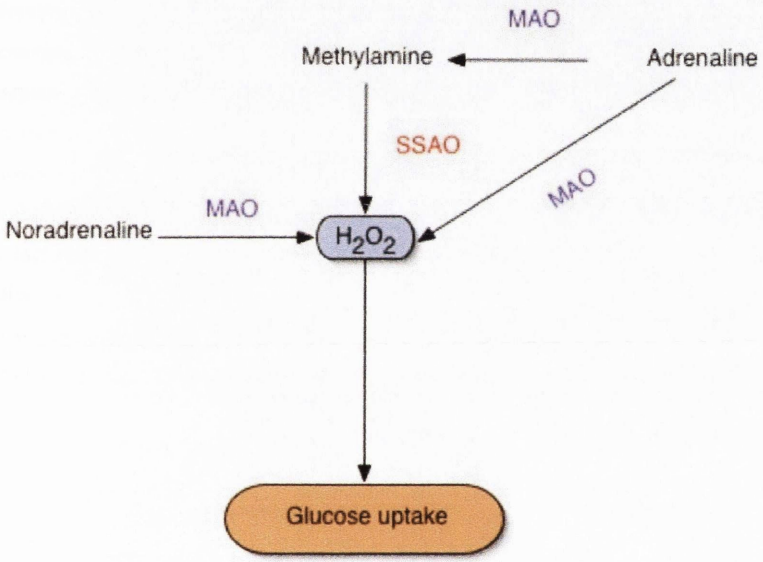


Fig. 5.8.3. Diagrammatic illustration of the proposed mechanism through which adrenaline and noradrenaline may mediate an increase in glucose uptake due to oxidative deamination by the amine oxidases SSAO and MAO.

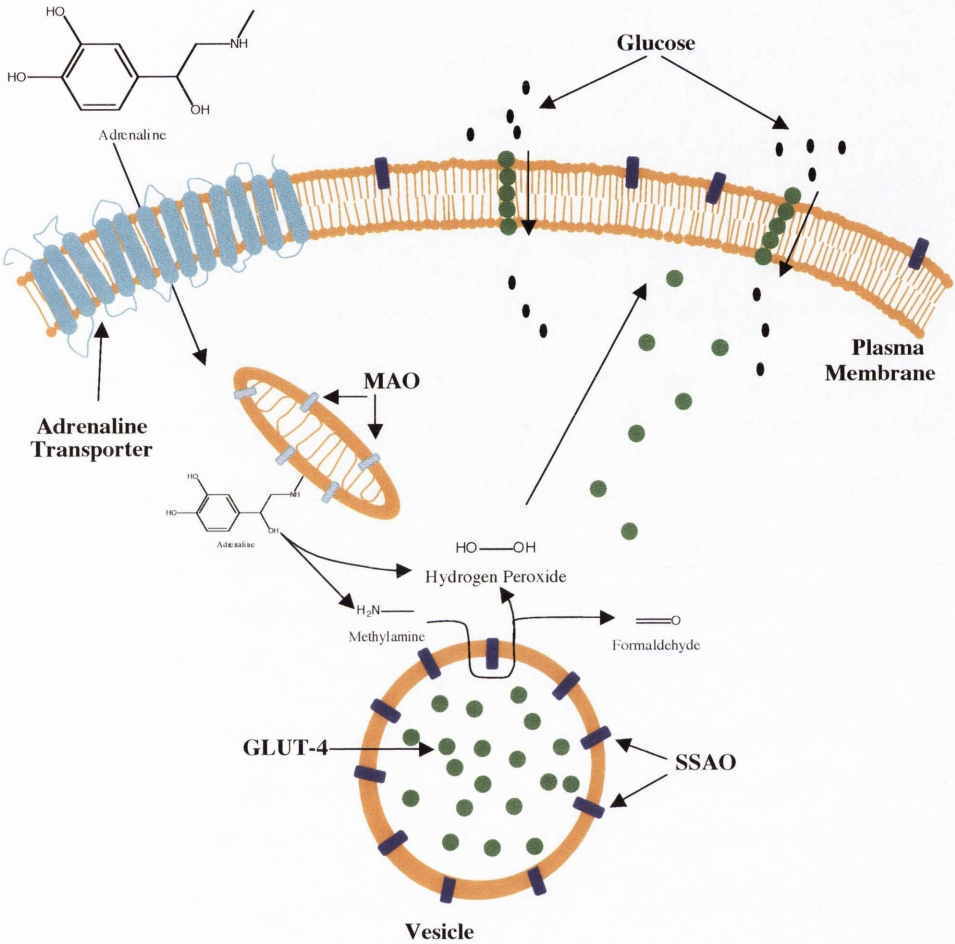
from the studies that showed that the α- and β-adrenergic receptor adrenergic antagonists appeared to cause a small reduction of the effects of adrenaline but this did not reach significance. The observation that the uptake inhibitor, maprotiline, abolished the (-)-adrenaline-stimulation of glucose uptake supports this finding. More importantly, it indicates that it is necessary for these hormones to enter the cell in order exert the stimulatory effects seen in this study. The stimulation by both adrenaline and noradrenaline was abolished by inhibition of MAO, indicating their oxidation by this enzyme, with the liberation of hydrogen peroxide, to be involved. The large reduction of the adrenaline effect, but not that of noradrenaline by the SSAO inhibitor, semicarbazide, shows that enzyme to be important in producing hydrogen peroxide by oxidising the methylamine liberated by MAO. The GLUT4-vesicular localisation of SSAO, as opposed to the mitochondrial location of MAO, together with the higher amounts of SSAO in adipocytes might suggest that it plays the dominant role in GLUT4 mobilisation. However, studies over a range of adrenaline concentrations would be necessary to show whether this was, indeed, the case under physiological conditions.

Adrenaline and noradrenaline are both involved in the sympathic regulation of white adipose tissue (WAT). High concentrations of noradrenaline released from sympathetic nerves stimulate β3-adrenoreceptors in rat WAT leading to the mobilisation of fatty acids as an energy source for other tissues, but inhibits the secretion of the hormone leptin, and has variable effects on a number of tissue factors and cytokines. The adrenal medulla secretes mainly adrenaline into the circulation an important function of which is to raise blood glucose by stimulating liver β2-receptors. Circulating adrenaline levels are not high enough to affect WAT adipose β3-receptors, but sufficient adrenaline may be secreted when stimulated by a fall in plasma glucose (Giacobino, 1996). Perhaps it is under such conditions that this metabolism-based effect of adrenaline glucose uptake may become important.

In summary, adrenaline, which is a MAO substrate, gives rise to methylamine upon metabolism and therefore produces a substrate for SSAO. Hydrogen peroxide formed in both the MAO- and SSAO-catalysed reactions is involved in the increased glucose

uptake, presumably *via* GLUT4 mobilisation. In contrast, stimulation of glucose uptake in the presence of noradrenaline is mediated by MAO alone. A schematic representation of this mechanism is outlined in Fig 5.8.3.

Fig. 5.8.3. Proposed mechanism through which SSAO can utilise MAO-derived methylamine as a substrate and therefore result in both enzymes playing a role in mediating the translocation of GLUT4-containing vesicles to the cell surface, which results in an increase in glucose uptake.


HO
HO
NH
OH
Adrenaline
Glucose
Plasma
Membrane
MAO
Adrenaline
Transporter
Adrenaline
HO—OH
Hydrogen Peroxide
H2N—
Methylamine
=O
Formaldehyde
GLUT-4
SSAO
Vesicle

Chapter Six

General Discussion

6.1 Discussion

In recent years the roles of the amine oxidase family of enzymes have become more distinct. The differentiation of the copper/TPQ and FAD containing enzymes has allowed for targeted in-depth studies on each group. Most interesting results appear to emanate from investigation into the multiple functions attributed to the SSAO branch of amine oxidases, which appear as elusive as they are interesting (see Fig. 6.1). One of the products of the oxidative deamination of amines by amine oxidases, H_2O_2, now appears to have a significant role in many biological mechanisms and is now seen as a second messenger in many situations such as in the activation of NF kappa B (Winrow *et al.*, 1993).

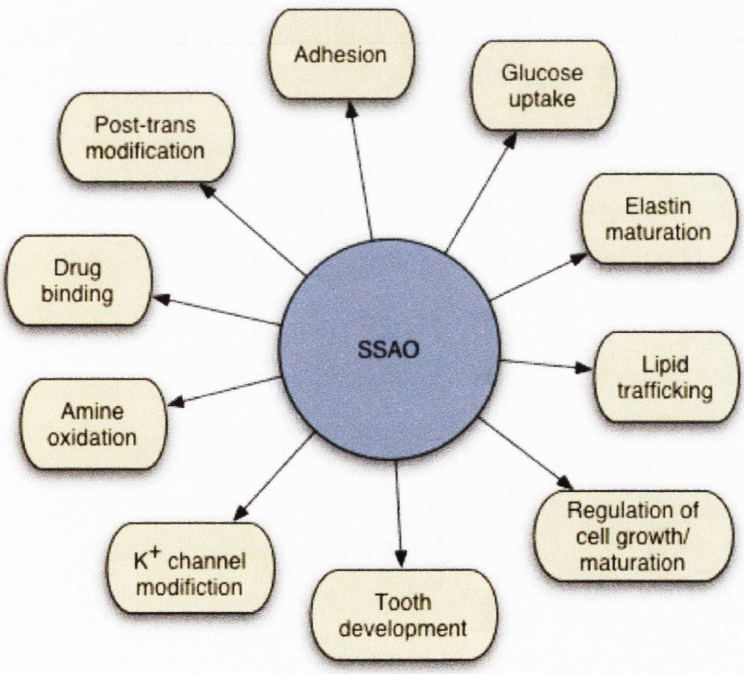


Fig. 6.1 The multiple functions of SSAO

H_2O_2 is also involved in the inflammatory response and is released in large amounts by granulocytes, neutrophils and macrophages in order to combat invading pathogens (Baeuerle *et al.*, 1996). Further responses to injury mediated by H_2O_2 include the activation of tyrosine and serine–threonine kinases (Berk 1999) along with the activation of lymphocytes. It is, therefore, no surprise to see the involvement of amine-oxidase derived H_2O_2 plays such a substantial role in several of SSAO's functions such as GLUT4 regulation by SSAO (Enrique-Tarancón *et al.*, 1998), which is abolished once H_2O_2 is removed via the addition of catalase. The demonstration, in the present work, that adrenaline and noradrenaline must enter the adipocyte to stimulate GLU4 recruitment and that MAO inhibition prevents this effect, further supports the importance of H_2O_2 in this process.

This work has also elucidated a H_2O_2 based mechanism in another SSAO function, cell adhesion. H_2O_2 appears to function as a mediator of cell adhesion to possible ligands one of which may be amino sugars. Amino sugars are available in abundance on the cell surface usually in the form of molecules like Lewis antigens or part of a cell surface glycoprotein (Collins & Ferrier 1995). The role of H_2O_2 in the cell adhesion function appears to be reliant on its production via the SSAO-catalysed amine oxidase reaction and not from an external source, such as that normally associated with the inflammatory response. H_2O_2 is closely involved in allowing binding of the amino sugar within the active site of the enzyme, as described in Section 3.8. Although the majority of work reported here was using bovine plasma SSAO comparable results were obtained with a membrane-bound source of the enzyme (bovine lung). Comparisons of DNA sequences of both plasma and membrane-bound SSAO have shown that they are essentially the same enzyme with the membrane bound form attached to the cell membrane via an *N*-terminal region that is missing, due to proteolytic cleavage, from the plasma form (see Kurkijärvi *et al.*, 1998).

Increased levels of SSAO in several disease states, such as breast cancer (Lizcano *et al.*, 1991), liver disease (Kurkijärvi *et al.*, 1998) amongst others (reviewed by O'Sullivan *et al.*, 2004), may assist any inflammatory response by increasing the migration of cells to

the site of injury, if an amine substrate is also present to allow for the production of H_2O_2. Although histamine is released during inflammation, it is a very poor substrate for SSAO from most sources (see Raimondi *et al.*, 1997). Indeed the activity towards this substrate is often difficult to measure at physiological pH values. However, since, in the mechanism proposed in the present work, it is the H_2O_2 generated in the vicinity of the enzyme active site that is necessary for adhesion, a poor substrate may indeed be able to function in this way. Furthermore, it would minimise the production of the potentially toxic products, aldehyde and NH_3, which may exacerbate the conditions (see Yu 1998), at sensitive sites.

The increased levels of SSAO in some disease states may modulate the vascular adhesion action of the enzyme. Modulation may be a reflection of the utilization of amine substrates by the plasma form of the enzyme rather than the membrane bound form. However disease states where plasma SSAO levels are decreased are likely to require a vascular adhesion mechanism, for example burns (see Lyles, 1996) and breast cancer (Lizcano *et al.*,1991) to allow repair and defense actions to take place.

Interestingly, two of the novel synthetic amino sugars studied showed concentration-dependent but not time-dependent inhibition of SSAO. The compound DD6B, which is the most structurally similar to a general amino sugar and the simplest of the synthesised, proved to be an effective inhibitor and might form a starting point for the development of SSAO-inhibitory drugs. Furthermore, more detailed studies of these compounds and their analogues, in comparison with the amino sugars, such as galactosamine, may help to explain the interactions that result in the H_2O_2-dependent inhibition.

Drug binding, originally reported as an ability to bind amiloride by both SSAO and MAO by amine oxidases now appears to be a common occurrence (Novonty *et al.*, 1994). This work shows that hydroxyzine, another commonly used drug, also binds to and inhibits both SSAO and MAO. The normal uses of hydroxyzine are as an antihistamine (Panayotopoulos & Panayotopoulos 1990), as an anaesthetic (Silver & Codesmith 1980) or in the treatment in neurosis (Pishkin & Shurley 1983). Interactions with either SSAO

or MAO have not been previously reported, but should be taken into consideration when assessing the behaviour of these drugs. Clearly the binding might affect its availability and inhibition of the amine oxidases may contribute to its effectiveness or side-effects. Although inhibitors are effective in conditions such as anxiety, this is a specific property of MAO-A inhibition and hydroxyzine was shown to be a poor inhibitor of that isoenzyme. Intriguingly, the membrane bound SSAO was only inhibited by higher hydroxyzine concentrations than the plasma form (mM compared to μM). This may show a further distinction between what are essentially the same enzyme, as has been previously shown for the interactions between plasma and membrane bound SSAO with amiloride and sensitivity to cyanide (K_m, 50 μM for membrane-bound SSAO, compared to 730 μM for plasma enzyme; Lizcano *et al.*, 1998).

The ability of hydroxyzine to inhibit mast cell migration into the brain in the EAE mouse model (Dimitriadou *et al.*, 2000) opens up interesting avenues of investigation. It is possible that the cell migration is reduced due to an inhibition of the vascular adhesion function of SSAO. Thus, the possible link to EAE progression and multiple sclerosis might be an intriguing addition to the ever-growing list of roles SSAO. In the present work, the SSAO inhibitor 2-BEA (2-bromoethylamine) was shown to prevent induction of EAE by MOG (myelin oligodendrocyte glycoprotein), as well as to reduce whole brain SSAO activity. Unfortunately, after the third treatment with 2-BEA an, unforeseen, toxicity effect resulted in a high mortality rate. An alternative, and more potent, SSAO inhibitor, methylhydrazine, reduced SSAO activity in a number of tissues, including brain, but showed no ability in reducing MOG-induced EAE, possibly due to the freely reversible nature of the inhibition. However, a 100% mortality rate after the second dose of methylhydrazine made it hard to compare these effects of the two test inhibitors. Modulation of the progression of EAE does however seem a plausible function for SSAO, screening for a non lethal inhibitor of SSAO with the right characteristics would appear to be the next step. Of course, care is of the utmost importance as many SSAO inhibitors can also inactivate MAO. Compounds such as those in the 2-phenyl-3-haloallylamine series while good SSAO inhibitors will inhibit MAO (Lyles *et al.*, 1987). Species variation will also have to be taken into consideration in identifying such a

compound B24 (3,5-diethoxy-4-aminomethylpyridine), while highly selective for SSAO from some species and with only a weak inhibitory effect on MAO, shows extensive species variation (Fitzgerald, 1998 thesis).

SSAO is involved in regulating the translocation of the glucose transporter GLUT-4 and the resulting increase in glucose uptake in the presence of substrates such as tyramine (Marti *et al.*, 1998) and benzylamine (Enrique-Tarancón *et al.*, 1998). Further analysis showed that the action of SSAO and MAO oxidation on amine substrates also mimics the effects of insulin stimulation on adipocyte differentiation in 3T3 F442A cells, where both benzylamine and tyramine treatment produced morphologically typical adipocytes, accompanied by the stimulation of protein synthesis and triacylglyerol accumulation (Fontana *et al.*, 2001). However, benzylamine is a non-physiological amine and also a MAO-B substrate and tyramine non physiological is a preferential substrate for MAO A & B. An intriguing question is what might be the physiological substrate for such a mechanism. Adrenaline would appear to act as a possible source of the physiological SSAO substrate methylamine, after oxidation by MAO. The present results confirm that that the hydrogen peroxide produced in the MAO-catalysed reactions may have a role to play in GLUT-4 translocation and, possibly, in adipocyte maturation. MAO substrates have been shown to stimulate glucose uptake, albeit not at the same level as SSAO substrates (Mercier *et al.*, 2001). This may reflect the differing cellular locations of the two enzymes or their relative activities. A proportion of SSAO in adipocytes has been shown to be associated with the intracellular vesicles containing the GLUT-4 transporter (Enrique-Tarancón *et al.*, 1998), which may suggest a more significant association with the mechanism of GLUT-4 translocation than that of MAO, from which the H_2O_2 formed would have to travel from the mitochondrial outer membrane to reach the GLUT4 vesicles. Whatever the relative importance of these two amine oxidases, adrenaline would appear to be a strong candidate since, after transport into the cell, its metabolism generates H_2O_2 at two sites. Furthermore it will not be degraded by SSAO located at the cell surface.

SSAO appears to be a somewhat overworked protein and it is reasonable to assume that there will be competition between the different and ever growing list of functions that are attributed to it. Such competition may be exacerbated by exposure to xenobiotics that are substrates for the enzyme. SSAO is one of a growing number of proteins that have apparently disparate functions (see Tipton *et al.*, 2003). Ultimately quantitative analysis will be necessary to determine how these proteins may divide their time between their different functions under different physiological and pathological conditions. In contrast MAO, despite acting as a drug-binding protein and as a source of H_2O_2, it is often, perhaps unfairly considered to be rather single-minded in its dedication to amine oxidation. Indeed, more attention has been devoted to it as a possible source of H_2O_2, and thence reactive-oxygen containing radicals under pathological conditions (see, e.g., Kunduzova *et al.*, 2002). There have been various suggestions that the hydrogen peroxide formed in the MAO catalysed reaction may be used for other processes, including thyroxine formation in the thyroid (Kraiem *et al.*, 1989) and prostaglandin synthesis (Seregi *et al.*, 1982), but utilisation of products from one enzyme reaction for other processes are a central feature of cellular metabolism.

Elucidation of the functional roles of SSAO and MAO, including cross talk between them and the possible modulation of one by the other, is a matter of great potential interest for further study. Such work should be facilitated by the recent determinations of the crystal structures of the enzymes, MAO A (Ma *et al.*, 2004), MAO B (Binda *et al.* 2002) and VAP-1/SSAO (Airenne *et al.*, 2003 to be published), as well as the availability of these proteins through cloning technologies.

6.2 Future Studies

The avenues of further research in amine oxidases appears boundless, especially when focusing on SSAO. To further the work studied here several approaches can be taken in regard to each of the topics.

Adhesion

While inhibition was evident with amino sugars no effect was observed with their *N*-acetyl derivatives, however many of the glycosyl ligands involved in cellular recognition are essentially 'capped' by *N*-acetylation. Therefore, the operation of an adhesion mechanism, such as that proposed in this work may require the removal of these "caps". An interesting avenue to explore is the possible role of a deacetylase in this system, which would allow the progression of SSAO/VAP-1 adhesion process by removing the *N*-acetyl cap. There has been rather little reported work on the behaviour of such deacetylases in mammalian systems.

Most studies on the adhesion function of SSAO/VAP-1 have relied on semiquantitative, microscopy-based assays. The development of a qualitative cell-adhesion assay would facilitate further studies by allowing precise measurements of the effectiveness and higher throughput.

Mediation of vascular adhesion by amino sugars appears to be important, although the possibility that the amino sugars are simply analogues of other cell-surface molecules that act *in vivo* requires further study. However, glucosamine is now freely available and quite widely used as a health supplement. It is normal sold in a preparations containing chondroitin and targeted towards individuals suffering from joint pain and arthritis. As shown in this study, it does inhibit SSAO/VAP-1 in a similar way to galactosamine. Thus, consumption of glucosamine may be expected to interfere with the normal adhesion function of SSAO/VAP-1. Further studies would be necessary to show whether

such a process is involved in the reported beneficial effects of dietary glucosamine supplementation.

Drug binding

Further analysis of the interactions of amine oxidases with drugs, such as hydroxyzine, are required to show whether such interactions relate to the effectiveness of the drugs. Any consequences of the inhibition, or activation, of amine oxidases by such compounds should also be investigated. A systematic screening of commonly-prescribed and structurally-feasible target drugs should be undertaken. The development of a high-throughput assay procedure, would facilitate such studies. Results from such studies would allow for a greater understanding of the pharmacological behaviour of such drugs and, perhaps, reveal more detail regarding the substrate recognition and catalytic mechanism of these enzymes.

Allergic encephalomyelitis (EAE)

The observation that SSAO appears to have a role in EAE/MS progression is potentially exciting. However, the toxicity of the SSAO inhibitors used in this study was a problem. Screening of SSAO inhibitors is required to try and isolate a non-lethal/toxic inhibitor which has a workable physiological half life and can influence the progression of the disease.

Glucose transport

The role of SSAO and MAO in glucose-transporter regulation needs further clarification. Although it is shown in this study that adrenaline can act as a source for methylamine resulting in further H_2O_2 production to complement that produced from MAO oxidation of adrenaline, the question still exists as to whether this is the only important physiological substrate in this process would be beneficial to the understanding of this SSAO function. Use of a cell line, such as the 3T3 F442A cell line, which has been used

in other studies on GLUT-4/glucose uptake, may facilitate further work. The advantages of using a cell line would include easier manipulation and more quantitative analysis. They would also represent a much cleaner system as isolated adipocytes are likely to contain contaminants in the form of collagen and blood products, no matter how rigorous the preparation. Receptor desensitisation was also a problem with isolated adipocytes, which, hopefully, would be less severe with a cell-culture based system.

Of course, the use of isolated adipocytes is essential for elucidation of the actual physiological role and the importance of glucose regulation in diseases, such as diabetes mellitus. Therefore, studies with adipose tissue from a range of diabetic and obese patients, along with appropriately-matched controls may expand our knowledge of these conditions and show whether treatment regimes based on modulation of SSAO-mediated glucose transport might be a useful therapeutic strategy.

6.3 Concluding remarks

The amine oxidase family is now considered a diverse and multifunctional group of enzymes. Dependent on location and form (membrane or plasma) they regulated or influence a significant number of physiological functions. This study has contributed to our ever-expanding knowledge of these enzymes, in particularly SSAO. The cell adhesion function of SSAO has been shown to be mediated via its amine oxidase function. The drug-binding capabilities have been enlarged and these studies led to interesting observations on the possible role of SSAO in EAE/MS development, which is probably linked to its adhesion function. Expansion of the role of SSAO in glucose transport with the, physiologically realistic, substrate adrenaline may provide a basis for the fuller understanding of the actions of this hormone in glucose regulation.

Although MAO is not generally regarded as being such a multitalented protein as SSAO, it was also shown to contribute to the H_2O_2-dependent modulation of glucose uptake. As

with SSAO, its drug-binding capabilities were also extended to include, a previously unknown, inhibitory interaction with the drug hydroxyzine.

Appendix

Appendix I

	Enzyme Number	Enzyme Name
1.	**1.1.1.136**	UDP-N-acetylglucosamine 6-dehydrogenase
2.	**1.1.1.158**	UDP-N-acetylmuramate dehydrogenase
3.	**1.1.1.233**	N-acylmannosamine 1-dehydrogenase
4.	**1.14.18.2**	CMP-N-acetylneuraminate monooxygenase
5.	**1.6.2.2**	Cytochrome-b5 reductase
6.	**2.3.1.157**	Glucosamine-1-phosphate N-acetyltransferase
7.	**2.3.1.3**	Glucosamine N-acetyltransferase
8.	**2.3.1.4**	Glucosamine 6-phosphate N-acetyltransferase
9.	**2.3.1.96**	Glycoprotein N-palmitoyltransferase
10.	**2.4.1.16**	Chitin synthase
11.	**2.5.1.56**	N-acetylneuraminate synthase
12.	**2.5.1.57**	N-acylneuraminate-9-phosphate synthase
13.	**2.5.1.7**	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
14.	**2.6.1.16**	Glutamine-fructose-6-phosphate transaminase (isomerizing)
15.	**2.7.1.1**	Hexokinase; hexokinase type IV glucokinase
16.	**2.7.1.59**	N-acetylglucosamine kinase
17.	**2.7.1.60**	N-acylmannosamine kinase
18.	**2.7.1.69**	Protein-N(pi)-phosphohistidine-sugar phosphotransferase
19.	**2.7.1.8**	Glucosamine kinase
20.	**2.7.7.23**	UDP-N-acetylglucosamine diphosphorylase
21.	**2.7.7.43**	N-acylneuraminate cytidylyltransferase
22.	**3.1.3.-**	No such entry
23.	**3.1.3.29**	N-acylneuraminate-9-phosphatase
24.	**3.1.4.-**	No such entry
25.	**3.2.1.-**	No such entry
26.	**3.2.1.132**	Chitosanase
27.	**3.2.1.14**	Chitinase
28.	**3.2.1.52**	Beta-N-acetylhexosaminidase
29.	**3.5.1.25**	N-acetylglucosamine-6-phosphate deacetylase
30.	**3.5.1.33**	N-acetylglucosamine deacetylase
31.	**3.5.1.41**	Chitin deacetylase
32.	**3.5.99.6**	Glucosamine-6-phosphate deaminase
33.	**4.1.3.3**	N-acetylneuraminate lyase
34.	**5.1.3.14**	UDP-N-acetylglucosamine 2-epimerase
35.	**5.1.3.7**	UDP-N-acetylglucosamine 4-epimerase
36.	**5.1.3.8**	N-acylglucosamine 2-epimerase
37.	**5.1.3.9**	N-acylglucosamine-6-phosphate 2-epimerase
38.	**5.4.2.10**	Phosphoglucosamine mutase
39.	**5.4.2.3**	Phosphoacetylglucosamine mutase

Bibliography

Abella, A., Marti, L., Camps, M., Claret, M., Fernandez-Alvarez, J., Gomis, R., Guma, A., Viguerie, N., Carpene, C., Palacin, M., Testar, X. & Zorzano, A. (2003) Semicarbazide-sensitive amine oxidase/vascular adhesion protein-1 activity exerts an antidiabetic action in Goto-Kakizaki rats. Diabetes, 52, 1004-1013.

Abella, A., Marti, L., Carpene, C., Palacin, M., Testar, X. & Zorzano, A. (2003) Stimulation of glucose transport by semicarbazide-sensitive amine oxidase activity in adipocytes from diabetic rats. J Physiol Biochem, 59, 153-160.

af Klinteberg B. (1996) Biology, norms and personality: a developmental perspective. Neuropsychobiology, 34(3), 146-54

Airenne, T.T., Nymalm, Y. Kidron, H., Soderholm, A., Johnson, M.S., Salminen, T.A., (2003) Crystal structure of Human Vascular Adhesion Protein-1. To be published.

Aldrich, T.B. (1901) A preliminary report on the active principle of the suprarenal gland. Am J Physiol, 5, 457-461.

Alexopoulos, G.S., Lieberman, K.W., Frances, R. & Stokes, P.E. (1981) Platelet MAO during the alcohol withdrawal syndrome. Am J Psychiatry, 138, 1254-1255.

Alexopoulos, G.S., Lieberman, K.W. & Frances, R.J. (1983) Platelet MAO activity in alcoholic patients and their first-degree relatives. Am J Psychiatry, 140, 1501-1504.

Alm, P.O., Alm, M., Humble, K., Leppert, J., Sorensen, S., Lidberg, L. & Oreland, L. (1994) Criminality and platelet monoamine oxidase activity in former juvenile delinquents as adults. Acta Psychiatr Scand, 89, 41-45.

Andriopoulou, P., Navarro, P., Zanetti, A., Lampugnani, M.G. & Dejana, E. (1999) Histamine induces tyrosine phosphorylation of endothelial cell-to-cell adherens junctions. Arterioscler Thromb Vasc Biol, 19, 2286-2297.

Aoki, S., Manabe, T. & Okuyama, T. (1977) Molecular weight estimation of bovine brain mitochondrial monoamine oxidase. J Biochem (Tokyo), 82, 1533-1539.

Aytac, U., Claret, F.X., Ho, L., Sato, K., Ohnuma, K., Mills, G.B., Cabanillas, F., Morimoto, C. & Dang, N.H. (2001) Expression of CD26 and its associated dipeptidyl peptidase IV enzyme activity enhances sensitivity to doxorubicin-induced cell cycle arrest at the G(2)/M checkpoint. Cancer Res, 61, 7204-7210.

Baeuerle, P.A., Rupec, R.A. & Pahl, H.L. (1996) Reactive oxygen intermediates as

second messengers of a general pathogen response. Pathol Biol (Paris), 44, 29-35.

Baker, G.B., Urichuk, L.J., McKenna, K.F. & Kennedy, S.H. (1999) Metabolism of monoamine oxidase inhibitors. Cell Mol Neurobiol, 19, 411-426.

Banchelli, G., Buffoni, F. & Raimondi, L. (1988) On the presence of a clorgyline resistant benzylamine oxidase activity in mouse kidney. Pharmacol Res Commun, 20, 465-483.

Barrand, M.A. & Callingham, B.A. (1984) Solubilization and some properties of a semicarbazide-sensitive amine oxidase in brown adipose tissue of the rat. Biochem J, 222, 467-475.

Beck-Nielsen, H., Hother Nielsen, O., Damsbo, P., Vaag, A., Handberg, A. & Henriksen, J.E. (1990) Impairment of glucose tolerance: mechanism of action and impact on the cardiovascular system. Am J Obstet Gynecol, 163, 292-295.

Bellelli, A., Morpurgo, L., Mondovi, B. & Agostinelli, E. (2000) The oxidation and reduction reactions of bovine serum amine oxidase. A kinetic study. Eur J Biochem, 267, 3264-3269.

Bergeret, B., Blaschko, H. & Hawes, R. (1957) Occurrence of an amine oxidase in horse serum. Nature, 180, 1127-1128.

Berk, B.C. (1999) Redox signals that regulate the vascular response to injury. Thromb Haemost, 82, 810-817.

Berlin, I., Said, S., Spreux-Varoquaux, O., Olivares, R., Launay, J.M. & Puech, A.J. (1995) Monoamine oxidase A and B activities in heavy smokers. Biol Psychiatry, 38, 756-761.

Best C.H. (1929) The disappearance of histamine from autolysing lung tissue. J Physiol 67,356-263

Binda, C., Coda, A., Angelini, R., Federico, R., Ascenzi, P. & Mattevi, A. (1999) A 30-angstrom-long U-shaped catalytic tunnel in the crystal structure of polyamine oxidase. Structure Fold Des, 7, 265-276.

Binda, C., Hubalek, F., Li, M., Edmondson, D.E. & Mattevi, A. (2004) Crystal structure of human monoamine oxidase B, a drug target enzyme monotopically inserted into the mitochondrial outer membrane. FEBS Lett, 564, 225-228.

Binda, C., Hubalek, F., Li, M., Herzig, Y., Sterling, J., Edmondson, D.E. & Mattevi, A.

(2004) Crystal structures of monoamine oxidase B in complex with four inhibitors of the N-propargylaminoindan class. J Med Chem, 47, 1767-1774.

Binda, C., Newton-Vinson, P., Hubalek, F., Edmondson, D.E. & Mattevi, A. (2002) Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. Nat Struct Biol, 9, 22-26.

Birks, J. & Flicker, L. (2003) Selegiline for Alzheimer's disease. Cochrane Database Syst Rev, CD000442.

Blaschko H., Friedman P.J., Schlossman H. (1937) The oxidation of adrenaline and other amines. Biochem J 31,2187-2196

Blaschko H., Duthie R. (1945) Substrate specificities of amine oxidases of mammalian plasma. Biochem J 39,478-481

Blaschko, H., Friedman, P.J., Hawes, R. & Nilsson, K. (1959) The amine oxidases of mammalian plasma. J Physiol, 145, 384-404.

Bleeker-Wagemakers, L.M., Friedrich, U., Gal, A., Wienker, T.F., Warburg, M. & Ropers, H.H. (1985) Close linkage between Norrie disease, a cloned DNA sequence from the proximal short arm, and the centromere of the X chromosome. Hum Genet, 71, 211-214.

Bohnsack, B.L. & Hirschi, K.K. (2004) Nutrient regulation of cell cycle progression. Annu Rev Nutr, 24, 433-453.

Boix, F., Qiao, S.W., Kolpus, T. & Sagvolden, T. (1998) Chronic L-deprenyl treatment alters brain monoamine levels and reduces impulsiveness in an animal model of Attention-Deficit/Hyperactivity Disorder. Behav Brain Res, 94, 153-162.

Bono, P., Jalkanen, S. & Salmi, M. (1999) Mouse vascular adhesion protein 1 is a sialoglycoprotein with enzymatic activity and is induced in diabetic insulitis. Am J Pathol, 155, 1613-1624.

Bono, P., Salmi, M., Smith, D.J. & Jalkanen, S. (1998) Cloning and characterization of mouse vascular adhesion protein-1 reveals a novel molecule with enzymatic activity. J Immunol, 160, 5563-5571.

Boomsma, F., van Dijk, J., Bhaggoe, U.M., Bouhuizen, A.M. & van den Meiracker, A.H. (2000) Variation in semicarbazide-sensitive amine oxidase activity in plasma and tissues of mammals. Comp Biochem Physiol C Toxicol Pharmacol, 126, 69-78.

Boomsma, F., van Veldhuisen, D.J., de Kam, P.J., Man in't Veld, A.J., Mosterd, A., Lie, K.I. & Schalekamp, M.A. (1997) Plasma semicarbazide-sensitive amine oxidase is elevated in patients with congestive heart failure. Cardiovasc Res, 33, 387-391.

Boomsma, F., van Woerkens, L.J., Man in 't Veld, A.J., Verdouw, P.D. & Schalekamp, M.A. (1993) High activity of semicarbazide-sensitive amine oxidase (SSAO): an important source of errors in the determination of the concentration of dopamine in pig plasma. J Cardiovasc Pharmacol, 22, 198-202.

Boor, P.J. (1985) Allylamine cardiovascular toxicity: V. Tissue distribution and toxicokinetics after oral administration. Toxicology, 35, 167-177.

Boor, P.J. & Ferrans, V.J. (1985) Ultrastructural alterations in allylamine cardiovascular toxicity. Late myocardial and vascular lesions. Am J Pathol, 121, 39-54.

Boor, P.J. & Hysmith, R.M. (1987) Allylamine cardiovascular toxicity. Toxicology, 44, 129-145.

Boor, P.J., Trent, M.B., Lyles, G.A., Tao, M. & Ansari, G.A. (1992) Methylamine metabolism to formaldehyde by vascular semicarbazide-sensitive amine oxidase. Toxicology, 73, 251-258.

Bose, A., Cherniack, A.D., Langille, S.E., Nicoloro, S.M., Buxton, J.M., Park, J.G., Chawla, A. & Czech, M.P. (2001) G(alpha)11 signaling through ARF6 regulates F-actin mobilization and GLUT4 glucose transporter translocation to the plasma membrane. Mol Cell Biol, 21, 5262-5275.

Boulenger, J.P. & Uhde, T.W. (1983) [Treatment of panic attacks]. Encephale, 9, 299B-305B.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72, 248-254.

Brazeau, B.J., Johnson, B.J. & Wilmot, C.M. (2004) Copper-containing amine oxidases. Biogenesis and catalysis; a structural perspective. Arch Biochem Biophys, 428, 22-31.

Brok, H.P., Bauer, J., Jonker, M., Blezer, E., Amor, S., Bontrop, R.E., Laman, J.D. & t Hart, B.A. (2001) Non-human primate models of multiple sclerosis. Immunol Rev, 183, 173-185.

Brown, D.E., McGuirl, M.A., Dooley, D.M., Janes, S.M., Mu, D. & Klinman, J.P. (1991) The organic functional group in copper-containing amine oxidases. Resonance Raman spectra are consistent with the presence of topa quinone (6-hydroxydopa quinone) in the active site. J Biol Chem, 266, 4049-4051.

Brown, G.K. (2000) Glucose transporters: structure, function and consequences of deficiency. J Inherit Metab Dis, 23, 237-246.

Bucht, G., Adolfsson, R., Lithner, F. & Winblad, B. (1983) Changes in blood glucose and insulin secretion in patients with senile dementia of Alzheimer type. Acta Med Scand, 213, 387-392.

Buffoni, F. & Blaschko, H. (1964) Benzylamine Oxidase and Histaminase: Purification and Crystallization of an Enzyme from Pig Plasma. Proc R Soc Lond B Biol Sci, 161, 153-167.

Buffoni, F. & Cambi, S. (1990) A method for the isolation and identification of pyridoxal phosphate in proteins. Anal Biochem, 187, 44-50.

Buffoni, F., Cambi, S., Moneti, G. (1992) Pyrroloquinoline quinone, a method for its isolation and identification by mass spectrometry. Biooochim Biophys Acta, 1116(3), 297-304.

Cai, D. & Klinman, J.P. (1994) Copper amine oxidase: heterologous expression, purification, and characterization of an active enzyme in Saccharomyces cerevisiae. Biochemistry, 33, 7647-7653.

Callingham, B.A., Crosbie, A.E. & Rous, B.A. (1995) Some aspects of the pathophysiology of semicarbazide-sensitive amine oxidase enzymes. Prog Brain Res, 106, 305-321.

Carlo, P., Violani, E., Del Rio, M., Olasmaa, M., Santagati, S., Maggi, A. & Picotti, G.B. (1996) Monoamine oxidase B expression is selectively regulated by dexamethasone in cultured rat astrocytes. Brain Res, 711, 175-183.

Carlsson, A. Lindqvist, M., Magnusson T., Walbeck B. (1959) On the presence of 3-hydroxytyrptamine in the brain. Science, 471-473.

Carpene, C., Visentin, V., Morin, N., Prevot, D., Smih, F., Rouet, P., Jayat, D., Fontana, E. & Lizcano, J.M. (2003) Characterization of semicarbazide-sensitive amine oxidase in human subcutaneous adipocytes and search for novel functions.

Inflammopharmacology, 11, 119-126.

Casacchia, M. & Rossi, A. (1989) A comparison of moclobemide and imipramine in treatment of depression. Pharmacopsychiatry, 22, 152-155.

Castello, A., Rodriguez-Manzaneque, J.C., Camps, M., Perez-Castillo, A., Testar, X., Palacin, M., Santos, A. & Zorzano, A. (1994) Perinatal hypothyroidism impairs the normal transition of GLUT4 and GLUT1 glucose transporters from fetal to neonatal levels in heart and brown adipose tissue. Evidence for tissue-specific regulation of GLUT4 expression by thyroid hormone. J Biol Chem, 269, 5905-5912.

Cesura, A.M., Muggli-Maniglio, D., Lang, G., Imhof, R. & Da Prada, M. (1990) Monoamine oxidase inhibition by moclobemide and 2-amino-ethyl carboxamide derivatives: mode of action and kinetic characteristics. J Neural Transm Suppl, 32, 165-170.

Charcot, J.M., (1868) Histologie de la sclerose en plaques. Gazette des hopitaux, Paris, 41, 554-555.

Chen, D., Elmendorf, J.S., Olson, A.L., Li, X., Earp, H.S. & Pessin, J.E. (1997) Osmotic shock stimulates GLUT4 translocation in 3T3L1 adipocytes by a novel tyrosine kinase pathway. J Biol Chem, 272, 27401-27410.

Chen, D., Fucini, R.V., Olson, A.L., Hemmings, B.A. & Pessin, J.E. (1999) Osmotic shock inhibits insulin signaling by maintaining Akt/protein kinase B in an inactive dephosphorylated state. Mol Cell Biol, 19, 4684-4694.

Chen, Y., Nagpal, M.L. & Lin, T. (2003) Expression and regulation of glucose transporter 8 in rat Leydig cells. J Endocrinol, 179, 63-72.

Chen, Z.Y., Denney, R.M. & Breakefield, X.O. (1995) Norrie disease and MAO genes: nearest neighbors. Hum Mol Genet, 4 Spec No, 1729-1737.

Chernogubova, E., Cannon, B. & Bengtsson, T. (2004) Norepinephrine increases glucose transport in brown adipocytes via beta3-adrenoceptors through a cAMP, PKA, and PI3-kinase-dependent pathway stimulating conventional and novel PKCs. Endocrinology, 145, 269-280.

Chiba, K., Trevor, A. & Castagnoli, N., Jr. (1984) Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. Biochem Biophys Res Commun,

120, 574-578.

Christophe, B., Carlier, B., Gillard, M., Chatelain, P., Peck, M. & Massingham, R. (2003) Histamine H1 receptor antagonism by cetirizine in isolated guinea pig tissues: influence of receptor reserve and dissociation kinetics. Eur J Pharmacol, 470, 87-94.

Colivicchi, M.A., Raimondi, L., Bianchi, L., Tipton, K.F., Pirisino, R. & Della Corte, L. (2004) Taurine prevents streptozotocin impairment of hormone-stimulated glucose uptake in rat adipocytes. Eur J Pharmacol, 495, 209-215.

Collins, P.M. & Ferrier, R.J. (1995) Monosaccharides. Their Chemistry and Their Roles in Natural Products, J. Wiley and Sons, Chichester, UK.

Conforti, L., Raimondi, L. & Lyles, G.A. (1993) Metabolism of methylamine by semicarbazide-sensitive amine oxidase in white and brown adipose tissue of the rat. Biochem Pharmacol, 46, 603-607.

Conklin, D.J., Trent, M.B. & Boor, P.J. (1999) The role of plasma semicarbazide-sensitive amine oxidase in allylamine and beta-aminopropionitrile cardiovascular toxicity: mechanisms of myocardial protection and aortic medial injury in rats. Toxicology, 138, 137-154.

Craft, S., Asthana, S., Newcomer, J.W., Wilkinson, C.W., Matos, I.T., Baker, L.D., Cherrier, M., Lofgreen, C., Latendresse, S., Petrova, A., Plymate, S., Raskind, M., Grimwood, K. & Veith, R.C. (1999) Enhancement of memory in Alzheimer disease with insulin and somatostatin, but not glucose. Arch Gen Psychiatry, 56, 1135-1140.

Craft, S., Asthana, S., Schellenberg, G., Cherrier, M., Baker, L.D., Newcomer, J., Plymate, S., Latendresse, S., Petrova, A., Raskind, M., Peskind, E., Lofgreen, C. & Grimwood, K. (1999) Insulin metabolism in Alzheimer's disease differs according to apolipoprotein E genotype and gender. Neuroendocrinology, 70, 146-152.

Da Prada, M., Kettler, R., Keller, H.H., Cesura, A.M., Richards, J.G., Saura Marti, J., Muggli-Maniglio, D., Wyss, P.C., Kyburz, E. & Imhof, R. (1990) From moclobemide to Ro 19-6327 and Ro 41-1049: the development of a new class of reversible, selective MAO-A and MAO-B inhibitors. J Neural Transm Suppl, 29,

279-292.

Davidson, J., McLeod, M.N. & White, H.L. (1978) Inhibition of platelet monoamine oxidase in depressed subjects treated with phenelzine. Am J Psychiatry, 135, 470-472.

Davison, A.N. (1956) Pyridoxal phosphate as coenzyme of diamine oxidase. Biochem J, 64, 546-548.

Day, T. & Greenfield, S.A. (2002) A non-cholinergic, trophic action of acetylcholinesterase on hippocampal neurones in vitro: molecular mechanisms. Neuroscience, 111, 649-656.

de la Chapelle, A., Sankila, E.M., Lindlof, M., Aula, P. & Norio, R. (1985) Norrie disease caused by a gene deletion allowing carrier detection and prenatal diagnosis. Clin Genet, 28, 317-320.

Deckert, J., Catalano, M., Syagailo, Y.V., Bosi, M., Okladnova, O., Di Bella, D., Nothen, M.M., Maffei, P., Franke, P., Fritze, J., Maier, W., Propping, P., Beckmann, H., Bellodi, L. & Lesch, K.P. (1999) Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum Mol Genet, 8, 621-624.

Den Boer, J.A., van Vliet, I.M. & Westenberg, H.G. (1995) Recent developments in the psychopharmacology of social phobia. Eur Arch Psychiatry Clin Neurosci, 244, 309-316.

Deng, Y. & Yu, P.H. (1999) Assessment of the deamination of aminoacetone, an endogenous substrate for semicarbazide-sensitive amine oxidase. Anal Biochem, 270, 97-102.

Dimitriadou, V., Pang, X. & Theoharides, T.C. (2000) Hydroxyzine inhibits experimental allergic encephalomyelitis (EAE) and associated brain mast cell activation. Int J Immunopharmacol, 22, 673-684.

Donnelly, D., Overington, J.P. & Blundell, T.L. (1994) The prediction and orientation of alpha-helices from sequence alignments: the combined use of environment-dependent substitution tables, Fourier transform methods and helix capping rules. Protein Eng, 7, 645-653.

Dooley, D.M. & Cote, C.E. (1984) Inactivation of beef plasma amine oxidase by sulfide.

J Biol Chem, 259, 2923-2926.

Dooley, D.M., McGuirl, M.A., Brown, D.E., Turowski, P.N., McIntire, W.S. & Knowles, P.F. (1991) A Cu(I)-semiquinone state in substrate-reduced amine oxidases. Nature, 349, 262-264.

Dove, J.E., Schwartz, B., Williams, N.K. & Klinman, J.P. (2000) Investigation of spectroscopic intermediates during copper-binding and TPQ formation in wild-type and active-site mutants of a copper-containing amine oxidase from yeast. Biochemistry, 39, 3690-3698.

Ducluzeau, P.H., Fletcher, L.M., Vidal, H., Laville, M. & Tavare, J.M. (2002) Molecular mechanisms of insulin-stimulated glucose uptake in adipocytes. Diabetes Metab, 28, 85-92.

Duine, J.A. & Frank, J. (1981) Quinoprotein alcohol dehydrogenase from a non-methylotroph, Acinetobacter calcoaceticus. J Gen Microbiol, 122, 201-209.

Ebadi, M., Sharma, S., Shavali, S. & El Refaey, H. (2002) Neuroprotective actions of selegiline. J Neurosci Res, 67, 285-289.

Edmondson, D.E., Mattevi, A., Binda, C., Li, M. & Hubalek, F. (2004) Structure and mechanism of monoamine oxidase. Curr Med Chem, 11, 1983-1993.

Edwards, D.J., Spiker, D.G., Kupfer, D.J., Foster, G., Neil, J.F. & Abrams, L. (1978) Platelet monoamine oxidase in affective disorders. Arch Gen Psychiatry, 35, 1443-1446.

Ehringer, H. & Hornykiewicz, O. (1960) [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system.]. Klin Wochenschr, 38, 1236-1239.

Eisler, T., Teravainen, H., Nelson, R., Krebs, H., Weise, V., Lake, C.R., Ebert, M.H., Whetzel, N., Murphy, D.L., Kopin, I.J. & Calne, D.B. (1981) Deprenyl in Parkinson disease. Neurology, 31, 19-23.

El Hadri, K., Moldes, M., Mercier, N., Andreani, M., Pairault, J. & Feve, B. (2002) Semicarbazide-sensitive amine oxidase in vascular smooth muscle cells: differentiation-dependent expression and role in glucose uptake. Arterioscler Thromb Vasc Biol, 22, 89-94.

Elliott, J. & Callingham, B.A. (1991) Effect of benzylamine and its metabolites on the

responses of the isolated perfused mesenteric arterial bed of the rat. J Auton Pharmacol, 11, 323-335.

Elliott, J., Callingham, B.A. & Sharman, D.F. (1989) Metabolism of amines in the isolated perfused mesenteric arterial bed of the rat. Br J Pharmacol, 98, 507-514.

Emilsson, L., Saetre, P., Balciuniene, J., Castensson, A., Cairns, N. & Jazin, E.E. (2002) Increased monoamine oxidase messenger RNA expression levels in frontal cortex of Alzheimer's disease patients. Neurosci Lett, 326, 56-60.

Enrique-Tarancon, G., Castan, I., Morin, N., Marti, L., Abella, A., Camps, M., Casamitjana, R., Palacin, M., Testar, X., Degerman, E., Carpene, C. & Zorzano, A. (2000) Substrates of semicarbazide-sensitive amine oxidase co-operate with vanadate to stimulate tyrosine phosphorylation of insulin-receptor-substrate proteins, phosphoinositide 3-kinase activity and GLUT4 translocation in adipose cells. Biochem J, 350 Pt 1, 171-180.

Enrique-Tarancon, G., Marti, L., Morin, N., Lizcano, J.M., Unzeta, M., Sevilla, L., Camps, M., Palacin, M., Testar, X., Carpene, C. & Zorzano, A. (1998) Role of semicarbazide-sensitive amine oxidase on glucose transport and GLUT4 recruitment to the cell surface in adipose cells. J Biol Chem, 273, 8025-8032.

Faintrenie, G. & Geloen, A. (1998) Alpha-1 adrenergic stimulation of glucose uptake in rat white adipocytes. J Pharmacol Exp Ther, 286, 607-610.

Farnsworth, P.N., Schleich, T.W., Groth-Vasselli, B. & Kuckel, C.L. (1988) Studies on the molecular organization of the lens. Ophthalmic Res, 20, 126-131.

Feldstein, A., Hoagland, H., Rivera Oktem, M. & Freeman, H. (1965) MAO inhibition and antidepressant activity. Int J Neuropsychiatry, 1, 384-387.

Fisman, M. & Watt, V.M. (1986) Investigation of the polymorphic region in the 5' flanking region of the insulin gene in patients with Alzheimer's disease. Can J Neurol Sci, 13, 471-474.

Fitzgerald, D (1998) Studies on the inhibition and modulationof amine oxidase activities. PhD thesis, Trinity College, Dublin.

Fontana, E., Boucher, J., Marti, L., Lizcano, J.M., Testar, X., Zorzano, A. & Carpene, C. (2001) Amine oxidase substrates mimic several of the insulin effects on adipocyte differentiation in 3T3 F442A cells. Biochem J, 356, 769-777.

Forster-Hovarth, C., Dome, B., Paku, S., Ladanyi, A., Somlai, B., Jalkanen, S. & Timar, J. (2004) Loss of vascular adhesion protein-1 expression in intratumoral microvessels of human skin melanoma. Melanoma Res, 2, 135-40.

Fowler, J.S., Logan, J., Wang, G.J. & Volkow, N.D. (2003) Monoamine oxidase and cigarette smoking. Neurotoxicology, 24, 75-82.

Fowler, J.S., Volkow, N.D., Wang, G.J., Pappas, N., Logan, J., MacGregor, R., Alexoff, D., Shea, C., Schlyer, D., Wolf, A.P., Warner, D., Zezulkova, I. & Cilento, R. (1996) Inhibition of monoamine oxidase B in the brains of smokers. Nature, 379, 733-736.

Fowler, J.S., Volkow, N.D., Wang, G.J., Pappas, N., Logan, J., Shea, C., Alexoff, D., MacGregor, R.R., Schlyer, D.J., Zezulkova, I. & Wolf, A.P. (1996) Brain monoamine oxidase A inhibition in cigarette smokers. Proc Natl Acad Sci U S A, 93, 14065-14069.

Franot, C., Mabic., Castagnoli. Jr. N., (1998) Chemical model studies on the monoamine oxidase-B catalyzed oxidation of 4-substituted1-cyclopropyl-1,2,3,6-tetrahyropyridines. Biorganic & Medicinal Chemistry, 6,283-291.

Frelin, C., Vigne, P., Barbry, P. & Lazdunski, M. (1987) Molecular properties of amiloride action and of its Na+ transporting targets. Kidney Int, 32, 785-793.

Frolich, L., Blum-Degen, D., Riederer, P. & Hoyer, S. (1999) A disturbance in the neuronal insulin receptor signal transduction in sporadic Alzheimer's disease. Ann N Y Acad Sci, 893, 290-293.

Furlan, R., Brambilla, E., Ruffini, F., Poliani, P.L., Bergami, A., Marconi, P.C., Franciotta, D.M., Penna, G., Comi, G., Adorini, L. & Martino, G. (2001) Intrathecal delivery of IFN-gamma protects C57BL/6 mice from chronic-progressive experimental autoimmune encephalomyelitis by increasing apoptosis of central nervous system-infiltrating lymphocytes. J Immunol, 167, 1821-1829.

Gareri, P., Falconi, U., De Fazio, P. & De Sarro, G. (2000) Conventional and new antidepressant drugs in the elderly. Prog Neurobiol, 61, 353-396.

Garpenstrand, H., Ekblom, J., Backlund, L.B., Oreland, L. & Rosenqvist, U. (1999) Elevated plasma semicarbazide-sensitive amine oxidase (SSAO) activity in Type 2 diabetes mellitus complicated by retinopathy. Diabet Med, 16, 514-521.

Garpenstrand, H., Longato-Stadler, E., af Klinteberg, B., Grigorenko, E., Damberg, M., Oreland, L. & Hallman, J. (2002) Low platelet monoamine oxidase activity in Swedish imprisoned criminal offenders. Eur Neuropsychopharmacol, 12, 135-140.

Giacobino, J.P. (1996) Role of the beta3-adrenoceptor in the control of leptin expression. Horm Metab Res, 28, 633-637.

Gillard, M., van der Perren, C., Moguilevsky, N., Massingham, R., Chatelain, P. (2002) Binding characteristics of cetirizine and levocetirizine to human H_1 histamine receptors: contribution of Lys^{191} and Thr^{194}. Mol Pharmacology, 61, 392-399.

Giustizieri, M.L., Albanesi, C., Fluhr, J., Gisondi, P., Norgauer, J. & Girolomoni, G. (2004) H1 histamine receptor mediates inflammatory responses in human keratinocytes. J Allergy Clin Immunol, 114, 1176-1182.

Gokturk, C., Nordquist, J., Sugimoto, H., Forsberg-Nilsson, K., Nilsson, J. & Oreland, L. (2004) Semicarbazide-sensitive amine oxidase in transgenic mice with diabetes. Biochem Biophys Res Commun, 325, 1013-1020.

Goldwaser, I., Gefel, D., Gershonov, E., Fridkin, M. & Shechter, Y. (2000) Insulin-like effects of vanadium: basic and clinical implications. J Inorg Biochem, 80, 21-25.

Goodpaster, B.H. (2002) Measuring body fat distribution and content in humans. Curr Opin Clin Nutr Metab Care, 5, 481-487.

Gorman, J., Liebowitz, M.R., Fyer, A.J., Levitt, M., Baron, M., Davies, S. & Klein, D.F. (1985) Platelet monoamine oxidase activity in patients with panic disorder. Biol Psychiatry, 20, 852-857.

Gornall, A.G., Bardawill, C.J., David, M.M., (1949) Determination of seurm proteins by means of the Biuret reagent. J Biol Chem, 177, 751-766

Gronvall, J.L., Garpenstrand, H., Oreland, L. & Ekblom, J. (1998) Autoradiographic imaging of formaldehyde adducts in mice: possible relevance for vascular damage in diabetes. Life Sci, 63, 759-768.

Gronvall-Nordquist, J.L., Backlund, L.B., Garpenstrand, H., Ekblom, J., Landin, B., Yu, P.H., Oreland, L. & Rosenqvist, U. (2001) Follow-up of plasma semicarbazide-sensitive amine oxidase activity and retinopathy in Type 2 diabetes mellitus. J Diabetes Complications, 15, 250-256.

Hahn, T., Barth, S., Graf, R., Engelmann, M., Beslagic, D., Reul, J.M., Holsboer, F., Dohr, G. & Desoye, G. (1999) Placental glucose transporter expression is regulated by glucocorticoids. J Clin Endocrinol Metab, 84, 1445-1452.

Hare, M.L.C. (1928) Tyramine oxidase, a new enzyme system in the liver. Biochem J 22, 968-979

Hayes, B.E. & Clarke, D.E. (1990) Semicarbazide-sensitive amine oxidase activity in streptozotocin diabetic rats. Res Commun Chem Pathol Pharmacol, 69, 71-83.

Holmes, L.B. (1971) Norrie's disease--an x-linked syndrome of retinal malformation, mental retardation and deafness. N Engl J Med, 284, 367-368.

Holt, A. & Callingham, B.A. (1994) The ex vivo effects of procarbazine and methylhydrazine on some rat amine oxidase activities. J Neural Transm Suppl, 41, 439-443.

Holt, A. & Callingham, B.A. (1995) Further studies on the ex-vivo effects of procarbazine and monomethylhydrazine on rat semicarbazide-sensitive amine oxidase and monoamine oxidase activities. J Pharm Pharmacol, 47, 837-845.

Holt, A., Sharman, D.F., Baker, G.B. & Palcic, M.M. (1997) A continuous spectrophotometric assay for monoamine oxidase and related enzymes in tissue homogenates. Anal Biochem, 244, 384-392.

Holt, A., Sharman, D.F. & Callingham, B.A. (1992) Effects in-vitro of procarbazine metabolites on some amine oxidase activities in the rat. J Pharm Pharmacol, 44, 494-499.

Houen, G. (1999) Mammalian Cu-containing amine oxidases (CAOs): new methods of analysis, structural relationships, and possible functions. APMIS Suppl, 96, 1-46.

Hoyer, S., Henneberg, N., Knapp, S., Lannert, H. & Martin, E. (1996) Brain glucose metabolism is controlled by amplification and desensitization of the neuronal insulin receptor. Ann N Y Acad Sci, 777, 374-379.

Hoyer, S. & Nitsch, R. (1989) Cerebral excess release of neurotransmitter amino acids subsequent to reduced cerebral glucose metabolism in early-onset dementia of Alzheimer type. J Neural Transm, 75, 227-232.

Huang, R.H. & Eiduson, S. (1977) Significance of multiple forms of brain monoamine oxidase in situ as probed by electron spin resonance. J Biol Chem, 252, 284-290.

Hubalek, F., Binda, C., Khalil, A., Li, M., Mattevi, A., Castagnoli, N. & Edmondson, D.E. (2005) Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. J Biol Chem, 280, 15761-15766.

Hug, G. (1976) Glycogen storage diseases. Birth Defects Orig Artic Ser, 12, 145-175.

Iglesias-Osma, M.C., Garcia-Barrado, M.J., Visentin, V., Pastor-Mansilla, M.F., Bour, S., Prevot, D., Valet, P., Moratinos, J. & Carpene, C. (2004) Benzylamine exhibits insulin-like effects on glucose disposal, glucose transport, and fat cell lipolysis in rabbits and diabetic mice. J Pharmacol Exp Ther, 309, 1020-1028.

Imanaka, T., Hayashi, H., Kishi, K., Wang, L., Ishii, K., Hazeki, O., Katada, T. & Ebina, Y. (1998) Reconstitution of insulin signaling pathways in rat 3Y1 cells lacking insulin receptor and insulin receptor substrate-1. Evidence that activation of Akt is insufficient for insulin-stimulated glycogen synthesis or glucose uptake in rat 3Y1 cells. J Biol Chem, 273, 25347-25355.

Isakoff, S.J., Taha, C., Rose, E., Marcusohn, J., Klip, A. & Skolnik, E.Y. (1995) The inability of phosphatidylinositol 3-kinase activation to stimulate GLUT4 translocation indicates additional signaling pathways are required for insulin-stimulated glucose uptake. Proc Natl Acad Sci U S A, 92, 10247-10251.

Jaakkola, K., Jalkanen, S., Kaunismaki, K., Vanttinen, E., Saukko, P., Alanen, K., Kallajoki, M., Voipio-Pulkki, L.M. & Salmi, M. (2000) Vascular adhesion protein-1, intercellular adhesion molecule-1 and P-selectin mediate leukocyte binding to ischemic heart in humans. J Am Coll Cardiol, 36, 122-129.

Jaakkola, K., Kaunismaki, K., Tohka, S., Yegutkin, G., Vanttinen, E., Havia, T., Pelliniemi, L.J., Virolainen, M., Jalkanen, S. & Salmi, M. (1999) Human vascular adhesion protein-1 in smooth muscle cells. Am J Pathol, 155, 1953-1965.

James, D.E. (1994) Targeting of the insulin-regulatable glucose transporter (GLUT-4). Biochem Soc Trans, 22, 668-670.

Janes, S.M., Mu, D., Wemmer, D., Smith, A.J., Kaur, S., Maltby, D., Burlingame, A.L. & Klinman, J.P. (1990) A new redox cofactor in eukaryotic enzymes: 6-hydroxydopa at the active site of bovine serum amine oxidase. Science, 248, 981-987.

Janez, A., Worrall, D.S., Imamura, T., Sharma, P.M. & Olefsky, J.M. (2000) The osmotic shock-induced glucose transport pathway in 3T3-L1 adipocytes is mediated by gab-1 and requires Gab-1-associated phosphatidylinositol 3-kinase activity for full activation. J Biol Chem, 275, 26870-26876.

Jiang, S., Xin, R., Lin, S., Qian, Y., Tang, G., Wang, D. & Wu, X. (2001) Linkage studies between attention-deficit hyperactivity disorder and the monoamine oxidase genes. Am J Med Genet, 105, 783-788.

Jiang, S., Xin, R., Wu, X., Lin, S., Qian, Y., Ren, D., Tang, G. & Wang, D. (2000) Association between attention deficit hyperactivity disorder and the DXS7 locus. Am J Med Genet, 96, 289-292.

Joost, H.G. & Thorens, B. (2001) The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). Mol Membr Biol, 18, 247-256.

Kabat, E.A., Glusman, M., Knaub, V. (1948) Quantitative estimation of the albumin and gammaglobulin in normal and pathological cerebrospinal fluid by immunochemical methods. Amer J Med, 4, 653-62.

Kagan, H.M., Vaccaro, C.A., Bronson, R.E., Tang, S.S. & Brody, J.S. (1986) Ultrastructural immunolocalization of lysyl oxidase in vascular connective tissue. J Cell Biol, 103, 1121-1128.

Kanzaki, M., Watson, R.T., Artemyev, N.O. & Pessin, J.E. (2000) The trimeric GTP-binding protein (G(q)/G(11)) alpha subunit is required for insulin-stimulated GLUT4 translocation in 3T3L1 adipocytes. J Biol Chem, 275, 7167-7175.

Kim, J.M., Hoegy, S.E., Mariano, P.S., (1995) Flavin chemical models for monoamine oxidase inactivationby Cyclopropylamines, α-Silyamines and Hydrazines. J Am Chem Soc., 117, 100-105.

Klinman, J.P. (1996) New quinocofactors in eukaryotes. J Biol Chem, 271, 27189-27192.

Kochersperger, L.M., Parker, E.L., Siciliano, M., Darlington, G.J. & Denney, R.M. (1986) Assignment of genes for human monoamine oxidases A and B to the X chromosome. J Neurosci Res, 16, 601-616.

Koeslag, J.H., Saunders, P.T. & Terblanche, E. (2003) A reappraisal of the blood glucose homeostat which comprehensively explains the type 2 diabetes mellitus-syndrome

X complex. J Physiol, 549, 333-346.

Kozka, I.J., Clark, A.E., Reckless, J.P., Cushman, S.W., Gould, G.W. & Holman, G.D. (1995) The effects of insulin on the level and activity of the GLUT4 present in human adipose cells. Diabetologia, 38, 661-666.

Kraiem, Z., Sadeh, O. & Youdim, M.B. (1989) Monoamine oxidase activity and triiodothyronine biosynthesis in human cultured thyroid cells. Br J Pharmacol, 96, 465-469.

Kralik, S.F., Liu, P., Leffler, B.J. & Elmendorf, J.S. (2002) Ceramide and glucosamine antagonism of alternate signaling pathways regulating insulin- and osmotic shock-induced glucose transporter 4 translocation. Endocrinology, 143, 37-46.

Kumar, V., Dooley, D.M., Freeman, H.C., Guss, J.M., Harvey, I., McGuirl, M.A., Wilce, M.C. & Zubak, V.M. (1996) Crystal structure of a eukaryotic (pea seedling) copper-containing amine oxidase at 2.2 A resolution. Structure, 4, 943-955.

Kunduzova, O.R., Bianchi, P., Parini, A. & Cambon, C. (2002) Hydrogen peroxide production by monoamine oxidase during ischemia/reperfusion. Eur J Pharmacol, 448, 225-230.

Kupriyanova, T.A. & Kandror, K.V. (1999) Akt-2 binds to Glut4-containing vesicles and phosphorylates their component proteins in response to insulin. J Biol Chem, 274, 1458-1464.

Kurkijarvi, R., Adams, D.H., Leino, R., Mottonen, T., Jalkanen, S. & Salmi, M. (1998) Circulating form of human vascular adhesion protein-1 (VAP-1): increased serum levels in inflammatory liver diseases. J Immunol, 161, 1549-1557.

Kurkijarvi, R., Jalkanen, S., Isoniemi, H. & Salmi, M. (2001) Vascular adhesion protein-1 (VAP-1) mediates lymphocyte-endothelial interactions in chronic kidney rejection. Eur J Immunol, 31, 2876-2884.

Kwan, S.W. & Abell, C.W. (1992) cDNA cloning and sequencing of rat monoamine oxidase A: comparison with the human and bovine enzymes. Comp Biochem Physiol B, 102, 143-147.

Lader, M. & Scotto, J.C. (1998) A multicentre double-blind comparison of hydroxyzine, buspirone and placebo in patients with generalized anxiety disorder. Psychopharmacology (Berl), 139, 402-406.

Lan, N.C., Heinzmann, C., Gal, A., Klisak, I., Orth, U., Lai, E., Grimsby, J., Sparkes, R.S., Mohandas, T. & Shih, J.C. (1989) Human monoamine oxidase A and B genes map to Xp 11.23 and are deleted in a patient with Norrie disease. Genomics, 4, 552-559.

Langdown, M.L., Holness, M.J. & Sugden, M.C. (2001) Early growth retardation induced by excessive exposure to glucocorticoids in utero selectively increases cardiac GLUT1 protein expression and Akt/protein kinase B activity in adulthood. J Endocrinol, 169, 11-22.

Langford, S.D., Trent, M.B., Balakumaran, A. & Boor, P.J. (1999) Developmental vasculotoxicity associated with inhibition of semicarbazide-sensitive amine oxidase. Toxicol Appl Pharmacol, 155, 237-244.

Langford, S.D., Trent, M.B. & Boor, P.J. (2002) Semicarbazide-sensitive amine oxidase and extracellular matrix deposition by smooth-muscle cells. Cardiovasc Toxicol, 2, 141-150.

Langston, J.W. (1985) MPTP neurotoxicity: an overview and characterization of phases of toxicity. Life Sci, 36, 201-206.

Lapierre, Y.D. (1982) Neurotransmitter functions in depression. Prog Neuropsychopharmacol Biol Psychiatry, 6, 639-644.

Lawrence, J.T. & Birnbaum, M.J. (2001) ADP-ribosylation factor 6 delineates separate pathways used by endothelin 1 and insulin for stimulating glucose uptake in 3T3-L1 adipocytes. Mol Cell Biol, 21, 5276-5285.

Lecrubier, Y. (1994) Risk-benefit assessment of newer versus older monoamine oxidase (MAO) inhibitors. Drug Saf, 10, 292-300.

Lee, A.D., Hansen, P.A. & Holloszy, J.O. (1995) Wortmannin inhibits insulin-stimulated but not contraction-stimulated glucose transport activity in skeletal muscle. FEBS Lett, 361, 51-54.

Lee, Y., Shepard, E., Smith, J., Dooley, D.M., Sayre, L.M. (2001) Catalytic turnover of substrate benzylamines by the quinon-dependent plasma amine oxidase leads to H_2O_2-dependent inactivation: evidence for generation of a cofactor-derived benzoxazole. Biochemistry, 40, 822-829.

Levy, E.R., Powell, J.F., Buckle, V.J., Hsu, Y.P., Breakefield, X.O. & Craig, I.W. (1989)

Localization of human monoamine oxidase-A gene to Xp11.23-11.4 by in situ hybridization: implications for Norrie disease. Genomics, 5, 368-370.

Lewinsohn, R. (1984) Mammalian monoamine-oxidizing enzymes, with special reference to benzylamine oxidase in human tissues. Braz J Med Biol Res, 17, 223-256.

Lewinsohn, R., Bohm, K., Glover, V. & Sandler, M. (1978) A benzylamine oxidase distinct from monoamine oxidase B--widespread distribution in man and rat. Biochem Pharmacol, 27, 1857-1863.

Li, J., Hu, X., Selvakumar, P., Russell, R.R., 3rd, Cushman, S.W., Holman, G.D. & Young, L.H. (2004) Role of the nitric oxide pathway in AMPK-mediated glucose uptake and GLUT4 translocation in heart muscle. Am J Physiol Endocrinol Metab, 287, E834-841.

Li, R., Chen, L., Cai, D., Klinman, J.P. & Mathews, F.S. (1997) Crystallographic study of yeast copper amine oxidase. Acta Crystallogr D Biol Crystallogr, 53, 364-370.

Lidberg, L., Modin, I., Oreland, L., Tuck, J.R. & Gillner, A. (1985) Platelet monoamine oxidase activity and psychopathy. Psychiatry Res, 16, 339-343.

Liu, L.B., Omata, W., Kojima, I. & Shibata, H. (2003) Insulin recruits GLUT4 from distinct compartments via distinct traffic pathways with differential microtubule dependence in rat adipocytes. J Biol Chem, 278, 30157-30169.

Lizcano, J.M., Balsa, D., Tipton, K.F. & Unzeta, M. (1991) The oxidation of dopamine by the semicarbazide-sensitive amine oxidase (SSAO) from rat vas deferens. Biochem Pharmacol, 41, 1107-1110.

Lizcano, J.M., Escrich, E., Ribalta, T., Muntane, J. & Unzeta, M. (1991) Amine oxidase activities in rat breast cancer induced experimentally with 7,12-dimethylbenz(alpha)anthracene. Biochem Pharmacol, 42, 263-269.

Lizcano, J.M., Fernandez de Arriba, A., Lyles, G.A. & Unzeta, M. (1994) Several aspects on the amine oxidation by semicarbazide-sensitive amine oxidase (SSAO) from bovine lung. J Neural Transm Suppl, 41, 415-420.

Lizcano, J.M., Fernandez de Arriba, A., Tipton, K.F. & Unzeta, M. (1996) Inhibition of bovine lung semicarbazide-sensitive amine oxidase (SSAO) by some hydrazine derivatives. Biochem Pharmacol, 52, 187-195.

Lizcano, J.M., Tipton, K.F. & Unzeta, M. (1998) Purification and characterization of

membrane-bound semicarbazide-sensitive amine oxidase (SSAO) from bovine lung. Biochem J, 331 (Pt 1), 69-78.

Lizcano, J.M., Unzeta, M. & Tipton, K.F. (2000) A spectrophotometric method for determining the oxidative deamination of methylamine by the amine oxidases. Anal Biochem, 286, 75-79.

Lu, G., Unge, T., Owera-Atepo, J.B., Shih, J.C., Ekblom, J. & Oreland, L. (1996) Characterization and partial purification of human monoamine oxidase-B expressed in Escherichia coli. Protein Expr Purif, 7, 315-322.

Lunelli, M., Di Paolo, M.L., Biadene, M., Calderone, V., Battistutta, R., Scarpa, M., Rigo, A. & Zanotti, G. (2005) Crystal structure of amine oxidase from bovine serum. J Mol Biol, 346, 991-1004.

Lyles, G.A. (1995) Substrate-specificity of mammalian tissue-bound semicarbazide-sensitive amine oxidase. Prog Brain Res, 106, 293-303.

Lyles, G.A. (1996) Mammalian plasma and tissue-bound semicarbazide-sensitive amine oxidases: biochemical, pharmacological and toxicological aspects. Int J Biochem Cell Biol, 28, 259-274.

Lyles, G.A. & Chalmers, J. (1992) The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. Biochem Pharmacol, 43, 1409-1414.

Lyles, G.A., Marshall, C.M., McDonald, I.A., Bey, P. & Palfreyman, M.G. (1987) Inhibition of rat aorta semicarbazide-sensitive amine oxidase by 2-phenyl-3-haloallylamines and related compounds. Biochem Pharmacol, 36, 2847-2853.

Lyles, G.A. & Singh, I. (1985) Vascular smooth muscle cells: a major source of the semicarbazide-sensitive amine oxidase of the rat aorta. J Pharm Pharmacol, 37, 637-643.

Ma, J., Kubota, F., Yoshimura, M., Yamashita, E., Nakagawa, A., Ito, A. & Tsukihara, T. (2004) Crystallization and preliminary crystallographic analysis of rat monoamine oxidase A complexed with clorgyline. Acta Crystallogr D Biol Crystallogr, 60, 317-319.

Macedo, T., Ribeiro, C.A., Cotrim, D., Tavares, P., Morgadinho, M.T., Caramona, M., Vicente, M.T., Rodrigues, L., Cardoso, M.G. & Keating, M.L. (1995)

Catecholamine and MHPG plasma levels, platelet MAO activity, and 3H-imipramine binding in heroin and cocaine addicts. Mol Neurobiol, 11, 21-29.

Markey, S.P., Johannessen, J.N., Chiueh, C.C., Burns, R.S. & Herkenham, M.A. (1984) Intraneuronal generation of a pyridinium metabolite may cause drug-induced parkinsonism. Nature, 311, 464-467.

Markwell, M.A., Haas, S.M., Bieber, L.L. & Tolbert, N.E. (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem, 87, 206-210.

Marti, L., Abella, A., Carpene, C., Palacin, M., Testar, X. & Zorzano, A. (2001) Combined treatment with benzylamine and low dosages of vanadate enhances glucose tolerance and reduces hyperglycemia in streptozotocin-induced diabetic rats. Diabetes, 50, 2061-2068.

Marti, L., Morin, N., Enrique-Tarancon, G., Prevot, D., Lafontan, M., Testar, X., Zorzano, A. & Carpene, C. (1998) Tyramine and vanadate synergistically stimulate glucose transport in rat adipocytes by amine oxidase-dependent generation of hydrogen peroxide. J Pharmacol Exp Ther, 285, 342-349.

Marttila, R.J., Rinne, U.K. & Tiilikainen, A. (1982) Virus antibodies in Parkinson's disease. Herpes simplex and measles virus antibodies in serum and CSF and their relation to HLA types. J Neurol Sci, 54, 227-238.

Mason, R.P., Olmstead, E.G. & Jacob, R.F. (2000) Antioxidant activity of the monoamine oxidase B inhibitor lazabemide. Biochem Pharmacol, 60, 709-716.

Mathys, K.C., Ponnampalam, S.N., Padival, S. & Nagaraj, R.H. (2002) Semicarbazide-sensitive amine oxidase in aortic smooth muscle cells mediates synthesis of a methylglyoxal-AGE: implications for vascular complications in diabetes. Biochem Biophys Res Commun, 297, 863-869.

Matosin-Matekalo, M., Mesonero, J.E., Laroche, T.J., Lacasa, M. & Brot-Laroche, E. (1999) Glucose and thyroid hormone co-regulate the expression of the intestinal fructose transporter GLUT5. Biochem J, 339 (Pt 2), 233-239.

Matsumoto, I., Staub, A., Benoist, C. & Mathis, D. (1999) Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. Science, 286, 1732-1735.

Matsuzawa, Y., Funahashi, T. & Nakamura, T. (1999) Molecular mechanism of

metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. Ann N Y Acad Sci, 892, 146-154.

Maycock, A.L., Abeles, R.H., Salach, J.I. & Singer, T.P. (1976) The structure of the covalent adduct formed by the interaction of 3-dimethylamino-1-propyne and the flavine of mitochondrial amine oxidase. Biochemistry, 15, 114-125.

Meadow, N.D., Fox, D.K. & Roseman, S. (1990) The bacterial phosphoenolpyruvate: glycose phosphotransferase system. Annu Rev Biochem, 59, 497-542.

Mellick, G.D., Buchanan, D.D., Silburn, P.A., Chan, D.K., Le Couteur, D.G., Law, L.K., Woo, J. & Pang, C.P. (2000) The monoamine oxidase B gene GT repeat polymorphism and Parkinson's disease in a Chinese population. J Neurol, 247, 52-55.

Meneilly, G.S. & Hill, A. (1993) Alterations in glucose metabolism in patients with Alzheimer's disease. J Am Geriatr Soc, 41, 710-714.

Mercier, N., Moldes, M., El Hadri, K. & Feve, B. (2001) Semicarbazide-sensitive amine oxidase activation promotes adipose conversion of 3T3-L1 cells. Biochem J, 358, 335-342.

Meszaros, Z., Szombathy, T., Raimondi, L., Karadi, I., Romics, L. & Magyar, K. (1999) Elevated serum semicarbazide-sensitive amine oxidase activity in non-insulin-dependent diabetes mellitus: correlation with body mass index and serum triglyceride. Metabolism, 48, 113-117.

Minogiannis, P., El-Mansoury, M., Betances, J.A., Sant, G.R. & Theoharides, T.C. (1998) Hydroxyzine inhibits neurogenic bladder mast cell activation. Int J Immunopharmacol, 20, 553-563.

Moldes, M., Feve, B. & Pairault, J. (1999) Molecular cloning of a major mRNA species in murine 3T3 adipocyte lineage. differentiation-dependent expression, regulation, and identification as semicarbazide-sensitive amine oxidase. J Biol Chem, 274, 9515-9523.

Morgan D.M. (1985) Polyamine oxidases. Biochen Soc Trans, 13, 322-6.

Morin, N., Lizcano, J.M., Fontana, E., Marti, L., Smih, F., Rouet, P., Prevot, D., Zorzano, A., Unzeta, M. & Carpene, C. (2001) Semicarbazide-sensitive amine oxidase substrates stimulate glucose transport and inhibit lipolysis in human adipocytes. J

Pharmacol Exp Ther, 297, 563-572.

Morin, N., Visentin, V., Calise, D., Marti, L., Zorzano, A., Testar, X., Valet, P., Fischer, Y. & Carpene, C. (2002) Tyramine stimulates glucose uptake in insulin-sensitive tissues in vitro and in vivo via its oxidation by amine oxidases. J Pharmacol Exp Ther, 303, 1238-1247.

Mu, D., Janes, S.M., Smith, A.J., Brown, D.E., Dooley, D.M. & Klinman, J.P. (1992) Tyrosine codon corresponds to topa quinone at the active site of copper amine oxidases. J Biol Chem, 267, 7979-7982.

Mu, D., Medzihradszky, K.F., Adams, G.W., Mayer, P., Hines, W.M., Burlingame, A.L., Smith, A.J., Cai, D. & Klinman, J.P. (1994) Primary structures for a mammalian cellular and serum copper amine oxidase. J Biol Chem, 269, 9926-9932.

Muchmore, D.B., Little, S.A. & de Haen, C. (1982) Counterregulatory control of intracellular hydrogen peroxide production by insulin and lipolytic hormones in isolated rat epididymal fat cells: a role of free fatty acids. Biochemistry, 21, 3886-3892.

Mueckler, M. (1994) Facilitative glucose transporters. Eur J Biochem, 219, 713-725.

Mueckler, M., Caruso, C., Baldwin, S.A., Panico, M., Blench, I., Morris, H.R., Allard, W.J., Lienhard, G.E. & Lodish, H.F. (1985) Sequence and structure of a human glucose transporter. Science, 229, 941-945.

Mure, M., Mills, S.A. & Klinman, J.P. (2002) Catalytic mechanism of the topa quinone containing copper amine oxidases. Biochemistry, 41, 9269-9278.

Murphy, D.L., Sims, K.B., Karoum, F., de la Chapelle, A., Norio, R., Sankila, E.M. & Breakefield, X.O. (1990) Marked amine and amine metabolite changes in Norrie disease patients with an X-chromosomal deletion affecting monoamine oxidase. J Neurochem, 54, 242-247.

Murray, J.M., Saysell, C.G., Wilmot, C.M., Tambyrajah, W.S., Jaeger, J., Knowles, P.F., Phillips, S.E. & McPherson, M.J. (1999) The active site base controls cofactor reactivity in Escherichia coli amine oxidase: x-ray crystallographic studies with mutational variants. Biochemistry, 38, 8217-8227.

Nakamura, S., Kawamata, T., Akiguchi, I., Kameyama, M., Nakamura, N. & Kimura, H. (1990) Expression of monoamine oxidase B activity in astrocytes of senile

plaques. Acta Neuropathol (Berl), 80, 419-425.

Nakatome, M., Tun, Z., Shimada, S. & Honda, K. (1998) Detection and analysis of four polymorphic markers at the human monoamine oxidase (MAO) gene in Japanese controls and patients with Parkinson's disease. Biochem Biophys Res Commun, 247, 452-456.

Nedergaard, J., Golozoubova, V., Matthias, A., Asadi, A., Jacobsson, A. & Cannon, B. (2001) UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. Biochim Biophys Acta, 1504, 82-106.

Nelson, T.J. & Boor, P.J. (1982) Allylamine cardiotoxicity--IV. Metabolism to acrolein by cardiovascular tissues. Biochem Pharmacol, 31, 509-514.

Nolte, L.A., Han, D.H., Hansen, P.A., Hucker, K.A. & Holloszy, J.O. (2003) A peroxovanadium compound stimulates muscle glucose transport as powerfully as insulin and contractions combined. Diabetes, 52, 1918-1925.

Nonogaki, K. (2000) New insights into sympathetic regulation of glucose and fat metabolism. Diabetologia, 43, 533-549.

Nordquist, J.E., Gokturk, C. & Oreland, L. (2002) Semicarbazide-sensitive amine oxidase (SSAO) gene expression in alloxan-induced diabetes in mice. Mol Med, 8, 824-829.

Norqvist, A., Oreland, L. & Fowler, C.J. (1982) Some properties of monoamine oxidase and a semicarbazide sensitive amine oxidase capable of the deamination of 5-hydroxytryptamine from porcine dental pulp. Biochem Pharmacol, 31, 2739-2744.

Novotny, W.F., Chassande, O., Baker, M., Lazdunski, M. & Barbry, P. (1994) Diamine oxidase is the amiloride-binding protein and is inhibited by amiloride analogues. J Biol Chem, 269, 9921-9925.

O'Sullivan, J., Unzeta, M., Healy, J., O'Sullivan, M.I., Davey, G. & Tipton, K.F. (2004) Semicarbazide-sensitive amine oxidases: enzymes with quite a lot to do. Neurotoxicology, 25, 303-315.

O'Sullivan, M., Tipton, K.F. & McDevitt, W.E. (2002) Immunolocalization of semicarbazide-sensitive amine oxidase in human dental pulp and its activity towards serotonin. Arch Oral Biol, 47, 399-406.

Obata, T. & Yamanaka, Y. (2000) Inhibition of monkey brain semicarbazide-sensitive amine oxidase by various antidepressants. Neurosci Lett, 286, 131-133.

Ochiai, Y., Itoh, K., Sakurai, E. & Tanaka, Y. (2005) Molecular cloning and characterization of rat semicarbazide-sensitive amine oxidase. Biol Pharm Bull, 28, 413-418.

Olanow, C.W. (1996) Deprenyl in the treatment of Parkinson's disease: clinical effects and speculations on mechanism of action. J Neural Transm Suppl, 48, 75-84.

Oreland, L., Fowler, C.J., Carlsson, A. & Magnusson, T. (1980) Monoamine oxidase -A and -B activity in the rat brain after hemitransection. Life Sci, 26, 139-146.

Oreland, L. & Gottfries, C.G. (1986) Brain and brain monoamine oxidase in aging and in dementia of Alzheimer's type. Prog Neuropsychopharmacol Biol Psychiatry, 10, 533-540.

Orsulak, P.J., Schildkraut, J.J., Schatzberg, A.F. & Herzog, J.M. (1978) Differences in platelet monoamine oxidase activity in subgroups of schizophrenic and depressive disorders. Biol Psychiatry, 13, 637-647.

Ott, A., Stolk, R.P., Hofman, A., van Harskamp, F., Grobbee, D.E. & Breteler, M.M. (1996) Association of diabetes mellitus and dementia: the Rotterdam Study. Diabetologia, 39, 1392-1397.

Ozelius, L., Hsu, Y.P., Bruns, G., Powell, J.F., Chen, S., Weyler, W., Utterback, M., Zucker, D., Haines, J., Trofatter, J.A. & et al. (1988) Human monoamine oxidase gene (MAOA): chromosome position (Xp21-p11) and DNA polymorphism. Genomics, 3, 53-58.

Panayotopoulos, S.M. & Panayotopoulou, E.S. (1990) Efficacy of cetirizine in the treatment of seasonal allergic rhinoconjunctivitis. Ann Allergy, 65, 146-148.

Parkinson J. (1817) An essay on the shaking plasy. London, Whittingham & Rowland.

Parsian, A. (1999) Sequence analysis of exon 8 of MAO-A gene in alcoholics with antisocial personality and normal controls. Genomics, 55, 290-295.

Parsons, M.R., Convery, M.A., Wilmot, C.M., Yadav, K.D., Blakeley, V., Corner, A.S., Phillips, S.E., McPherson, M.J. & Knowles, P.F. (1995) Crystal structure of a quinoenzyme: copper amine oxidase of Escherichia coli at 2 A resolution. Structure, 3, 1171-1184.

Pascual, J.M., Wang, D., Lecumberri, B., Yang, H., Mao, X., Yang, R. & De Vivo, D.C. (2004) GLUT1 deficiency and other glucose transporter diseases. Eur J Endocrinol, 150, 627-633.

Patek, D.R. & Hellerman, L. (1974) Mitochondrial monoamine oxidase. Mechanism of inhibition by phenylhydrazine and by aralkylhydrazines. Role of enzymatic oxidation. J Biol Chem, 249, 2373-2380.

Pekkel, V.A. & Kirkel, A.Z. (1985) [Subcellular localization, inhibiting specificity and catalytic properties of several aminooxidases from the human placenta]. Biokhimiia, 50, 289-299.

Pepper, J.P., Baumann, M.H., Ayestas, M. & Rothman, R.B. (2001) Inhibition of MAO-A fails to alter cocaine-induced increases in extracellular dopamine and norepinephrine in rat nucleus accumbens. Brain Res Mol Brain Res, 87, 184-189.

Perry, T. & Greig, N.H. (2002) The glucagon-like peptides: a new genre in therapeutic targets for intervention in Alzheimer's disease. J Alzheimers Dis, 4, 487-496.

Perry, T., Lahiri, D.K., Sambamurti, K., Chen, D., Mattson, M.P., Egan, J.M. & Greig, N.H. (2003) Glucagon-like peptide-1 decreases endogenous amyloid-beta peptide (Abeta) levels and protects hippocampal neurons from death induced by Abeta and iron. J Neurosci Res, 72, 603-612.

Pintar, J.E., Barbosa, J., Francke, U., Castiglione, C.M., Hawkins, M., Jr. & Breakefield, X.O. (1981) Gene for monoamine oxidase type A assigned to the human X chromosome. J Neurosci, 1, 166-175.

Pishkin, V. & Shurley, J.T. (1983) Doxepin and hydroxyzine in treating anxiety: effects of failure and motivation on cognitive performance. J Clin Psychiatry, 44, 57-59.

Portin, R. & Rinne, U.K. (1983) The effect of deprenyl (selegiline) on cognition and emotion in parkinsonian patients undergoing long-term levodopa treatment. Acta Neurol Scand Suppl, 95, 135-144.

Pugh, C.E.M. & Quastel J.H. (1937) Oxidation of aliphatic amines by brain and other tissues. Biochem J, 31,286-291

Raimondi, L., Banchelli, G., Ignesti, G., Pirisino, R. & Conforti, L. (1997) The histaminase activity of rat white adipocytes. Inflamm Res, 46, 125-131.

Raimondi, L., Conforti, L., Banchelli, G., Ignesti, G., Pirisino, R. & Buffoni, F. (1993)

Histamine lipolytic activity and semicarbazide-sensitive amine oxidase (SSAO) of rat white adipose tissue (WAT). Biochem Pharmacol, 46, 1369-1376.

Raimondi, L., Conforti, L., Banchelli, G., Sgromo, L. & Pirisino, R. (1997) Some histamine-related compounds interacting with the benzylamine-oxidizing activity of rat white adipocytes. J Pharm Pharmacol, 49, 542-550.

Raimondi, L., Pirisino, R., Ignesti, G., Banchelli, G. & Buffoni, F. (1992) A common distribution of the semicarbazide-sensitive amine oxidase activity (SSAO) in white adipose tissue (WAT). Pharmacol Res, 25 Suppl 1, 21-22.

Ramos, K., Grossman, S.L. & Cox, L.R. (1988) Allylamine-induced vascular toxicity in vitro: prevention by semicarbazide-sensitive amine oxidase inhibitors. Toxicol Appl Pharmacol, 95, 61-71.

Reinikainen, K.J., Paljarvi, L., Halonen, T., Malminen, O., Kosma, V.M., Laakso, M. & Riekkinen, P.J. (1988) Dopaminergic system and monoamine oxidase-B activity in Alzheimer's disease. Neurobiol Aging, 9, 245-252.

Riederer, P., Lachenmayer, L. & Laux, G. (2004) Clinical applications of MAO-inhibitors. Curr Med Chem, 11, 2033-2043.

Rigby, S.E., Hynson, R.M., Ramsay, R.R., Munro, A.W. & Scrutton, N.S. (2005) A stable tyrosyl radical in monoamine oxidase A. J Biol Chem, 280, 4627-4631.

Rinne, U.K. & Rinne, J.O. (1989) Treatment of early Parkinson's disease with controlled-release levodopa preparations. Neurology, 39, 78-81; discussion 95.

Ristow, M. (2004) Neurodegenerative disorders associated with diabetes mellitus. J Mol Med, 82, 510-529.

Rius, F.X., Knowles, P.F. & Pettersson, G. (1984) The kinetics of ammonia release during the catalytic cycle of pig plasma amine oxidase. Biochem J, 220, 767-772.

Rivers, T.M., Sprunt, D.H., Berry, G.P. (1933) Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. J Exp Med, 58, 39-53.

Roberts, C.K., Barnard, R.J., Scheck, S.H. & Balon, T.W. (1997) Exercise-stimulated glucose transport in skeletal muscle is nitric oxide dependent. Am J Physiol, 273, E220-225.

Ruggieri, S., Stocchi, F., Denaro, A., Baronti, F. & Agnoli, A. (1986) The role of MAO-b inhibitors in the treatment of Parkinson's disease. J Neural Transm Suppl, 22, 227-

233.

Salach, J.I. (1979) Monoamine oxidase from beef liver mitochondria: simplified isolation procedure, properties, and determination of its cysteinyl flavin content. Arch Biochem Biophys, 192, 128-137.

Salach, J.I., Singer, T.P., Castagnoli, N., Jr. & Trevor, A. (1984) Oxidation of the neurotoxic amine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by monoamine oxidases A and B and suicide inactivation of the enzymes by MPTP. Biochem Biophys Res Commun, 125, 831-835.

Salmi, M., Hellman, J. & Jalkanen, S. (1998) The role of two distinct endothelial molecules, vascular adhesion protein-1 and peripheral lymph node addressin, in the binding of lymphocyte subsets to human lymph nodes. J Immunol, 160, 5629-5636.

Salmi, M. & Jalkanen, S. (1992) A 90-kilodalton endothelial cell molecule mediating lymphocyte binding in humans. Science, 257, 1407-1409.

Salmi, M., Tohka, S., Berg, E.L., Butcher, E.C. & Jalkanen, S. (1997) Vascular adhesion protein 1 (VAP-1) mediates lymphocyte subtype-specific, selectin-independent recognition of vascular endothelium in human lymph nodes. J Exp Med, 186, 589-600.

Salmi, M., Tohka, S. & Jalkanen, S. (2000) Human vascular adhesion protein-1 (VAP-1) plays a critical role in lymphocyte-endothelial cell adhesion cascade under shear. Circ Res, 86, 1245-1251.

Salminen, T.A., Smith, D.J., Jalkanen, S. & Johnson, M.S. (1998) Structural model of the catalytic domain of an enzyme with cell adhesion activity: human vascular adhesion protein-1 (HVAP-1) D4 domain is an amine oxidase. Protein Eng, 11, 1195-1204.

Sandler, M., Carter, S.B., Cuthbert, M.F. & Pare, C.M. (1975) Is there an increase in monoamine-oxidase activity in depressive illness? Lancet, 1, 1045-1049.

Schmeideberg O (1878) Uber das verhaltniss des ammoniaks und das primaren monoaminbasen zur harnstoffbildung im theirlorper. Naunyn-Scheidebergs' Arch Pharmacol 8:1-14

Schultz, D.R., Barthal, J.S. & Garrett, G. (1977) Western equine encephalitis with rapid

onset of parkinsonism. Neurology, 27, 1095-1096.

Schulze, T.G., Muller, D.J., Krauss, H., Scherk, H., Ohlraun, S., Syagailo, Y.V., Windemuth, C., Neidt, H., Grassle, M., Papassotiropoulos, A., Heun, R., Nothen, M.M., Maier, W., Lesch, K.P. & Rietschel, M. (2000) Association between a functional polymorphism in the monoamine oxidase A gene promoter and major depressive disorder. Am J Med Genet, 96, 801-803.

Seregi, A., Serfozo, P., Mergl, Z. & Schaefer, A. (1982) On the mechanism of the involvement of monoamine oxidase in catecholamine-stimulated prostaglandin biosynthesis in particulate fraction of rat brain homogenates: role of hydrogen peroxide. J Neurochem, 38, 20-27.

Sharma, S.K., Carlson, E.C. & Ebadi, M. (2003) Neuroprotective actions of Selegiline in inhibiting 1-methyl, 4-phenyl, pyridinium ion (MPP+)-induced apoptosis in SK-N-SH neurons. J Neurocytol, 32, 329-343.

Shekim, W.O., Bylund, D.B., Alexson, J., Glaser, R.D., Jones, S.B., Hodges, K. & Perdue, S. (1986) Platelet MAO and measures of attention and impulsivity in boys with attention deficit disorder and hyperactivity. Psychiatry Res, 18, 179-188.

Shekim, W.O., Davis, L.G., Bylund, D.B., Brunngraber, E., Fikes, L. & Lanham, J. (1982) Platelet MAO in children with attention deficit disorder and hyperactivity: a pilot study. Am J Psychiatry, 139, 936-938.

Shih, J.C., Grimsby, J., Chen, K. & Zhu, Q.S. (1993) Structure and promoter organization of the human monoamine oxidase A and B genes. J Psychiatry Neurosci, 18, 25-32.

Shimizu, T., Nishihira, J., Watanabe, H., Abe, R., Ishibashi, T. & Shimizu, H. (2004) Cetirizine, an H1-receptor antagonist, suppresses the expression of macrophage migration inhibitory factor: its potential anti-inflammatory action. Clin Exp Allergy, 34, 103-109.

Siegel, R.C., Fu, J.C. & Chang, Y. (1976) Collagen cross-linking: the substrate specificity of lysyl oxidase. Adv Exp Med Biol, 74, 438-446.

Silver, H. & Codesmith, A.O. (1980) Anesthesia for outpatient head and neck aesthetic surgery. Ann Plast Surg, 5, 483-485.

Sillverman, R.B., (1995) Radical ideas about monoamine oxidase. Acc Chem Res, 28,

335-342.

Simpson, I.A., Yver, D.R., Hissin, P.J., Wardzala, L.J., Karnieli, E., Salans, L.B. & Cushman, S.W. (1983) Insulin-stimulated translocation of glucose transporters in the isolated rat adipose cells: characterization of subcellular fractions. Biochim Biophys Acta, 763, 393-407.

Sims, K.B., de la Chapelle, A., Norio, R., Sankila, E.M., Hsu, Y.P., Rinehart, W.B., Corey, T.J., Ozelius, L., Powell, J.F., Bruns, G. & et al. (1989) Monoamine oxidase deficiency in males with an X chromosome deletion. Neuron, 2, 1069-1076.

Sims, K.B., Ozelius, L., Corey, T., Rinehart, W.B., Liberfarb, R., Haines, J., Chen, W.J., Norio, R., Sankila, E., de la Chapelle, A. & et al. (1989) Norrie disease gene is distinct from the monoamine oxidase genes. Am J Hum Genet, 45, 424-434.

Singer, T.P., Salach, J.I., Castagnoli, N., Jr. & Trevor, A. (1986) Interactions of the neurotoxic amine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine with monoamine oxidases. Biochem J, 235, 785-789.

Sleeman, M.W., Zhou, H., Rogers, S., Ng, K.W. & Best, J.D. (1995) Retinoic acid stimulates glucose transporter expression in L6 muscle cells. Mol Cell Endocrinol, 108, 161-167.

Smith, D.J., Salmi, M., Bono, P., Hellman, J., Leu, T. & Jalkanen, S. (1998) Cloning of vascular adhesion protein 1 reveals a novel multifunctional adhesion molecule. J Exp Med, 188, 17-27.

Sobel, R.A., Blanchette, B.W., Bhan, A.K. & Colvin, R.B. (1984) The immunopathology of experimental allergic encephalomyelitis. I. Quantitative analysis of inflammatory cells in situ. J Immunol, 132, 2393-2401.

Sofuoglu, S., Dogan, P., Besim, T., Basturk, M. & Tanrikulu, G. (1992) MAO-B activity in depression and anxiety disorders. Biol Psychiatry, 31, 634-638.

Stalenheim, E.G. (2001) Relationships between attempted suicide, temperamental vulnerability, and violent criminality in a Swedish forensic psychiatric population. Eur Psychiatry, 16, 386-394.

Stefanis, C.N., Alevizos, B., Markianos, M. & Hatzimanolis, J. (1988) Effect of moclobemide on clinical and neurochemical variables in depressed patients

(preliminary findings). J Neural Transm Suppl, 26, 87-95.

Stillman, R.C., Wyatt, R.J., Murphy, D.L. & Rauscher, F.P. (1978) Low platelet monoamine oxidase activity and chronic marijuana use. Life Sci, 23, 1577-1581.

Stoff, D.M., Friedman, E., Pollock, L., Vitiello, B., Kendall, P.C. & Bridger, W.H. (1989) Elevated platelet MAO is related to impulsivity in disruptive behavior disorders. J Am Acad Child Adolesc Psychiatry, 28, 754-760.

Strolin Benedetti, M. (1982) [Biochemistry of depression and antidepressants]. Encephale, 8, 545-585.

Strolin Benedetti, M. & Dostert, P. (1987) Overview of the present state of MAO inhibitors. J Neural Transm Suppl, 23, 103-119.

Suarez-Merino, B., Bye, J., McDowall, J., Ross, M. & Craig, I.W. (2001) Sequence analysis and transcript identification within 1.5 MB of DNA deleted together with the NDP and MAO genes in atypical Norrie disease patients presenting with a profound phenotype. Hum Mutat, 17, 523.

Tabata, A., Takahashi, K., Masuko, S., Aizawa, H., Obata, T., Oreland, L. & Kinemuchi, H. (2003) Further evidence for suicide inhibition of semicarbazide-sensitive amine oxidase in guinea pig lung by 2-bromoethylamine. Methods Find Exp Clin Pharmacol, 25, 785-792.

Tabor, C.W., Tabor, H., Rosenthal, S.M. (1954) Purification of amine oxidase from beef plasma. J Biol Chem, 208, 645-661.

Taglialatela, M., Timmerman, H. & Annunziato, L. (2000) Cardiotoxic potential and CNS effects of first-generation antihistamines. Trends Pharmacol Sci, 21, 52-56.

Takagi, H., King, G.L. & Aiello, L.P. (1998) Hypoxia upregulates glucose transport activity through an adenosine-mediated increase of GLUT1 expression in retinal capillary endothelial cells. Diabetes, 47, 1480-1488.

Takahashi, S. (1977) Monoamine oxidase activity in blood platelets from manic and depressed patients. Folia Psychiatr Neurol Jpn, 31, 37-48.

Takahashi, S., Tani, N. & Yamane, H. (1976) Monoamine oxidase activity in blood platelets in alcoholism. Folia Psychiatr Neurol Jpn, 30, 455-462.

Takamine, J (1901) Adrenalin; the active principle of the suprarenal gland. Am J Pharm, 73, 523-531.

Tariot, P.N., Sunderland, T., Weingartner, H., Murphy, D.L., Welkowitz, J.A., Thompson, K. & Cohen, R.M. (1987) Cognitive effects of L-deprenyl in Alzheimer's disease. Psychopharmacology (Berl), 91, 489-495.

Taylor, M.E. & Drickamer, K. (2004) Introduction to glycobiology. Oxford Universty Press, Great Clarendon Street, Oxford, England.

Tejada-Simon, M.V., Zang, Y.C.Q., Yang, D., Hog, J., Li, S., Singh, R.A.K., Van den Berg-Loonen, E., Killian, J.M., Rivera. V.M., Zhang, J.Z. (2000) Aberrant T cell responses to myelin antigens during clinical exacerbation in patients with multiple sclerosis. International immunology, 12(12), 1641-1650.

Tipton, K.F. (1967) The presence of flavine-adenine dinucleotide in preparations of pig-brain monoamine oxidase. Biochem J, 104, 36P-37P.

Tipton, K.F. (1969) A sensitive fluorometric assay for monoamine oxidase. Anal Biochem, 28, 318-325.

Tipton, K.F. (1996) Patterns of enzyme inhibition, In: Enzymology LabFax (Edited by P.C. Engle) Bios Scienfific Publishers, Oxfor, UK & Academic Press, San Diego, USA.

Tipton, K.F., Davey, G., Motherway, M. (2000) Monoamine oxidase assays. Current Protocols in Pharmacology. Taylor, G.P., Sullivan, J.P. (Eds), 3.61-3.6.42

Tipton, K.F., Boyce, S., O'Sullivan, J., Davey, G.P. & Healy, J. (2004) Monoamine oxidases: certainties and uncertainties. Curr Med Chem, 11, 1965-1982.

Tipton, K.F., McCrodden, J.M. & Youdim, M.B. (1986) Oxidation and enzyme-activated irreversible inhibition of rat liver monoamine oxidase-B by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Biochem J, 240, 379-383.

Tipton, K.F. & Strolin Benedetti, M. (2001) In enzyme systems that metabolise drugs and other xenobiotics (loannides, C., ed), pp. 95-146, John Wiley & Sons, Chichester.

Tipton, K.F., O'Sullivan, M.I., Davey, G.P. & O'Sullivan, J. (2003) It can be a complicated life being an enzyme. Biochem Soc Trans, 31, 711-715.

Tohka, S., Laukkanen, M., Jalkanen, S. & Salmi, M. (2001) Vascular adhesion protein 1 (VAP-1) functions as a molecular brake during granulocyte rolling and mediates recruitment in vivo. Faseb J, 15, 373-382.

Vaiva, G., De Lenclave, M.B. & Bailly, D. (2002) Treatment of comorbid opiate

addiction and attention-deficit hyperactivity disorder (residual type) with moclobemide: a case report. Prog Neuropsychopharmacol Biol Psychiatry, 26, 609-611.

van Vliet, I.M., den Boer, J.A., Westenberg, H.G. & Slaap, B.R. (1996) A double-blind comparative study of brofaromine and fluvoxamine in outpatients with panic disorder. J Clin Psychopharmacol, 16, 299-306.

Visentin, V., Marq, P., Bour, S., Subra, C., Prevot, D., Morin, N., Valet, P., Monje, M.C., Nepveu, F. & Carpene, C. (2003) Effect of prolonged treatment with tyramine on glucose tolerance in streptozotocin-induced diabetic rats. J Physiol Biochem, 59, 225-232.

von Knorring, A.L., Bohman, M., von Knorring, L. & Oreland, L. (1985) Platelet MAO activity as a biological marker in subgroups of alcoholism. Acta Psychiatr Scand, 72, 51-58.

von Knorring, A.L., Hallman, J., von Knorring, L. & Oreland, L. (1991) Platelet monoamine oxidase activity in type 1 and type 2 alcoholism. Alcohol Alcohol, 26, 409-416.

Von Knorring, L. & Oreland, L. (1985) Personality traits and platelet monoamine oxidase in tobacco smokers. Psychol Med, 15, 327-334.

Von Korff R.V. (1977) Properties of mitochondrial monoamine oxidase isolated from rabbit brain and liver. Biokhimiia, 42(3), 396-402.

Waldmeier, P.C. (1987) Amine oxidases and their endogenous substrates (with special reference to monoamine oxidase and the brain). J Neural Transm Suppl, 23, 55-72.

Wang, X., Pietrangeli, P., Mateescu, M.A. & Mondovi, B. (1996) Extended substrate specificity of serum amine oxidase: possible involvement in protein posttranslational modification. Biochem Biophys Res Commun, 223, 91-97.

Wang, X.T., Dumoulin, M.J., Befani, O., Mondovi, B. & Mateescu, M.A. (1994) Joint chromatographic purification of bovine serum ceruloplasmin and amineoxidase. Prep Biochem, 24, 237-250.

Warburg, M. (1971) Norrie's disease. Birth Defects Orig Artic Ser, 7, 117-124.

Wei, Q., Yeung, M., Jurma, O.P. & Andersen, J.K. (1996) Genetic elevation of

monoamine oxidase levels in dopaminergic PC12 cells results in increased free radical damage and sensitivity to MPTP. J Neurosci Res, 46, 666-673.

Weinstein, S.P., O'Boyle, E., Fisher, M. & Haber, R.S. (1994) Regulation of GLUT2 glucose transporter expression in liver by thyroid hormone: evidence for hormonal regulation of the hepatic glucose transport system. Endocrinology, 135, 649-654.

Wender, P.H. (1998) Pharmacotherapy of attention-deficit/hyperactivity disorder in adults. J Clin Psychiatry, 59 Suppl 7, 76-79.

Weyler, W. (1989) Monoamine oxidase A from human placenta and monoamine oxidase B from bovine liver both have one FAD per subunit. Biochem J, 260, 725-729.

Wiberg, A. (1979) Increase in platelet monoamine oxidase activity during controlled abstinence after alcohol abuse. Med Biol, 57, 133-134.

Williams, C.H. & Lawson, J. (1998) Mechanism-based inhibition of monoamine oxidase by 3-aryl-Delta3-pyrrolines. Biochem J, 336 (Pt 1), 63-67.

Winrow, V.R., Winyard, P.G., Morris, C.J. & Blake, D.R. (1993) Free radicals in inflammation: second messengers and mediators of tissue destruction. Br Med Bull, 49, 506-522.

Winter, M.C., Kamath, A.M., Ries, D.R., Shasby, S.S., Chen, Y.T. & Shasby, D.M. (1999) Histamine alters cadherin-mediated sites of endothelial adhesion. Am J Physiol, 277, L988-995.

Wistow, G.J., Lietman, T., Williams, L.A., Stapel, S.O., de Jong, W.W., Horwitz, J. & Piatigorsky, J. (1988) Tau-crystallin/alpha-enolase: one gene encodes both an enzyme and a lens structural protein. J Cell Biol, 107, 2729-2736.

Wolfsdorf, J.I., Holm, I.A. & Weinstein, D.A. (1999) Glycogen storage diseases. Phenotypic, genetic, and biochemical characteristics, and therapy. Endocrinol Metab Clin North Am, 28, 801-823.

Wong, W.K., Chen, K. & Shih, J.C. (2001) Regulation of human monoamine oxidase B gene by Sp1 and Sp3. Mol Pharmacol, 59, 852-859.

Wong, W.K., Ou, X.M., Chen, K. & Shih, J.C. (2002) Activation of human monoamine oxidase B gene expression by a protein kinase C MAPK signal transduction pathway involves c-Jun and Egr-1. J Biol Chem, 277, 22222-22230.

Wood, I.S. & Trayhurn, P. (2003) Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br J Nutr, 89, 3-9.

Wouters, J. (1998) Structural aspects of monoamine oxidase and its reversible inhibition. Curr Med Chem, 5, 137-162.

Wright, E.M. (2001) Renal Na(+)-glucose cotransporters. Am J Physiol Renal Physiol, 280, F10-18.

Wright, E.M., Martin, M.G. & Turk, E. (2003) Intestinal absorption in health and disease--sugars. Best Pract Res Clin Gastroenterol, 17, 943-956.

Wright, E.M., Turk, E. & Martin, M.G. (2002) Molecular basis for glucose-galactose malabsorption. Cell Biochem Biophys, 36, 115-121.

Wu, H.F., Chen, K. & Shih, J.C. (1993) Site-directed mutagenesis of monoamine oxidase A and B: role of cysteines. Mol Pharmacol, 43, 888-893.

Wu, L., Mateescu, M.A., Wang, X.T., Mondovi, B. & Wang, R. (1996) Modulation of K+ channel currents by serum amineoxidase in neurons. Biochem Biophys Res Commun, 220, 47-52.

Xie, L., Helmerhorst, E., Taddei, K., Plewright, B., Van Bronswijk, W. & Martins, R. (2002) Alzheimer's beta-amyloid peptides compete for insulin binding to the insulin receptor. J Neurosci, 22, RC221.

Xu, L., Ma, J., Seigel, G.M. & Ma, J.X. (1999) l-Deprenyl, blocking apoptosis and regulating gene expression in cultured retinal neurons. Biochem Pharmacol, 58, 1183-1190.

Yadav K.D. & Knowles P.F. (1981) A catalytic mechanism for benzylamine oxidase from pig plasma. Eur J Biochem, 114, 133-138.

Yoo, B.Y. & Oreland, L. (1976) Electron microscopic localization of monoamine oxidase in the rat liver. Histochemistry, 46, 131-137.

Youdim, M.B. & Weinstock, M. (2002) Novel neuroprotective anti-Alzheimer drugs with anti-depressant activity derived from the anti-Parkinson drug, rasagiline. Mech Ageing Dev, 123, 1081-1086.

Young, S.N., Davis, B.A. & Gauthier, S. (1982) Precursors and metabolites of phenylethylamine, m and p-tyramine and tryptamine in human lumbar and cisternal cerebrospinal fluid. J Neurol Neurosurg Psychiatry, 45, 633-639.

Yu, P.H. (1998) Deamination of methylamine and angiopathy; toxicity of formaldehyde, oxidative stress and relevance to protein glycoxidation in diabetes. J Neural Transm Suppl, 52, 201-216.

Yu, P.H., Bowen, R.C., Davis, B.A. & Boulton, A.A. (1983) Platelet monoamine oxidase activity and trace acid levels in plasma of agoraphobic patients. Acta Psychiatr Scand, 67, 188-194.

Yu, P.H., Bowen, R.C., Davis, B.A. & Boulton, A.A. (1983) A study of the catabolism of trace amines in mentally disordered individuals with particular reference to agoraphobic patients with panic attacks. Prog Neuropsychopharmacol Biol Psychiatry, 7, 611-615.

Yu, P.H., Davis, B.A. & Deng, Y. (2001) 2-Bromoethylamine as a potent selective suicide inhibitor for semicarbazide-sensitive amine oxidase. Biochem Pharmacol, 61, 741-748.

Yu, P.H., Wang, M., Fan, H., Deng, Y. & Gubisne-Haberle, D. (2004) Involvement of SSAO-mediated deamination in adipose glucose transport and weight gain in obese diabetic KKAy mice. Am J Physiol Endocrinol Metab, 286, E634-641.

Yu, P.H. & Zuo, D.M. (1993) Oxidative deamination of methylamine by semicarbazide-sensitive amine oxidase leads to cytotoxic damage in endothelial cells. Possible consequences for diabetes. Diabetes, 42, 594-603.

Yu, P.H. & Zuo, D.M. (1997) Aminoguanidine inhibits semicarbazide-sensitive amine oxidase activity: implications for advanced glycation and diabetic complications. Diabetologia, 40, 1243-1250.

Zelechonok, Y., & Silverman, R.B., (1992) Silver(I)/Peroxydisulfate-induced oxidative decarboxylation of amino acids. A chemical model for a possible intermediate in the monoamine oxidase-catalyzed oxidation of amines. J Org Chem, 57,5787-5790.

Zeller E.A. (1938) Uber der enzymatischen abbau von histamin un diaminen. Helv Chim Acta, 21,881-890.

Zeller, E.A., Boshes, B., Arbit, J., Bieber, M., Blonsky, E.R., Dolkart, M. & Huprikar, S.V. (1976) Molecular biology of neurological and psychiatric disorders. I. Effect of parkinsonism, age, sex and L-dopa on platelet monoamine oxidase. J Neural

Transm, 39, 63-77.

Zhou, B.P., Lewis, D.A., Kwan, S.W. & Abell, C.W. (1995) Flavinylation of monoamine oxidase B. J Biol Chem, 270, 23653-23660.

Zhou, B.P., Lewis, D.A., Kwan, S.W., Kirksey, T.J. & Abell, C.W. (1995) Mutagenesis at a highly conserved tyrosine in monoamine oxidase B affects FAD incorporation and catalytic activity. Biochemistry, 34, 9526-9531.

Zhu, Q.S., Chen, K. & Shih, J.C. (1994) Bidirectional promoter of human monoamine oxidase A (MAO A) controlled by transcription factor Sp1. J Neurosci, 14, 7393-7403.

Zorzano, A., Abella, A., Marti, L., Carpene, C., Palacin, M. & Testar, X. (2003) Semicarbazide-sensitive amine oxidase activity exerts insulin-like effects on glucose metabolism and insulin-signaling pathways in adipose cells. Biochim Biophys Acta, 1647, 3-9.

Publications

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1647 (2003) 367–371
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

The inhibition of semicarbazide-sensitive amine oxidase by aminohexoses

Jeffrey O'Sullivan[a,*], Michael O'Sullivan[a], Keith F. Tipton[a], Mercedes Unzeta[b], Maria Del Mar Hernandez[b], Gavin P. Davey[a]

[a] *Department of Biochemistry, Trinity College, Dublin 2, Ireland*
[b] *Department of Biochemistry and Molecular Biology, Faculty of Medicine, Autonomous University of Barcelona, Bellaterra, Spain*

Received 14 July 2002; received in revised form 15 November 2002; accepted 22 January 2003

Abstract

Semicarbazide-sensitive amine oxidase (EC 1.4.3.6; amine:oxygen oxidoreductase (deaminating) (copper-containing); SSAO) is a multifunctional protein. It acts under inflammatory conditions as a vascular-adhesion protein (VAP-1), mediating the adhesion of lymphocytes to vascular endothelial cells. The relationships, if any, between this adhesion function and the enzymatic functions (amine-substrate specificity and catalysis) of SSAO have not yet been defined. Since cell surface amino sugars and their derivatives are known to be involved in cell-to-cell recognition, we have investigated their possible effects on the enzyme activity of SSAO. The aminohexoses galactosamine, glucosamine and mannosamine were not oxidatively deaminated by SSAO. However, their presence during the assay of benzylamine oxidation resulted in a time-dependent inhibition. This inhibition was shown to follow saturation kinetics with respect to hexosamine concentration. Although time-dependent, the inhibition of SSAO activity was found to be reversible by dilution. In contrast, there is no such inhibition when the *N*-acetylamino sugar derivatives or the parent sugars (galactose, glucose and mannose) replaced the amino sugars in the reaction mixture. These results suggest that the interactions between SSAO and aminohexoses are specific and, therefore, that the cell-adhesion functions and amine-recognition functions of VAP-1/SSAO may be interlinked.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Galactosamine; Inhibition; Vascular-adhesion protein 1 (VAP-1)

1. Introduction

The semicarbazide-sensitive amine oxidases (SSAO) are an ubiquitous group of enzymes found in plants, animals and microbes (see Refs. [1–4]). They belong to the class EC 1.4.3.6 (amine:oxygen oxidoreductase (deaminating) (copper-containing)) and contain TOPA (6-hydroxydopa; 2,4,5-trihydroxyphenylalanine) quinone as the redox cofactor (see Ref. [5]). They catalyse the overall reaction:

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + NH_3 + H_2O_2$$

In most mammals, the enzyme exists in tissue-bound and soluble (plasma) forms, but there are wide species and tissue differences in the activities [6]. The tissue-bound form contains a short intracellular domain, a single trans-membrane domain and a long extracellular domain that includes the catalytic site [7], which might suggest a scavenging or protective role towards circulating amines. However, a proportion of SSAO in adipocytes has been shown to be associated with the intracellular vesicles containing the GLUT4 glucose transporter [8].

There are large species differences in the specificities of SSAO, but the non-physiological amine benzylamine is a good substrate for the mammalian enzymes. The physiological substrates are believed to include aminoacetone [9] and methylamine [10], 2-phenylethylamine, tyramine [11] and dopamine [12]. Many of the substrates for SSAO are also oxidatively deaminated by the monoamine oxidases (EC 1.4.3.4, amine:oxygen oxidoreductase (deaminating) (flavine-containing); MAO), but aminoacetone and methylamine are not MAO substrates. SSAO also catalyses the oxidative deamination of a number of xenobiotics (see Ref. [13]). Although SSAO has been often regarded as being involved in the detoxification of amines, the products of the reaction are potentially more toxic than the amine substrates themselves (see Refs. [2,3]).

Abbreviations: MAO, monoamine oxidase; SSAO, semicarbazide-sensitive amine oxidase; VAP-1, vascular-adhesion protein 1

* Corresponding author.
E-mail address: josulli2@tcd.ie (J. O'Sullivan).

1570-9639/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1570-9639(03)00096-7

Several alternative functions for SSAO have been proposed. It has been suggested that one of the roles of SSAO is the formation of H_2O_2, which has an insulin-like effect in stimulating the transport of the GLUT4 glucose transporter to the cell-surface and inhibiting lipolysis in adipocytes [8,14]. Amine oxidation by SSAO has also been recently shown to stimulate glucose uptake by vascular smooth muscle cells [15] and to promote adipocyte differentiation [16]. It has also been reported that H_2O_2 may modulate the activity of SSAO [17,18]. Other, less clearly defined, functions that have been suggested include involvement in the post-translational modification of some proteins [19], cardioprotection [20] and modification of neuronal K^+-channel function [21]. SSAO is also an amiloride-binding protein (see Refs. [22,23]), although the significance, if any, of this is unclear.

Recently an adhesion protein, vascular-adhesion protein 1 (VAP-1) was found to have sequence homology with SSAO at the cDNA level [24]. This protein was subsequently found to possess SSAO activity. VAP-1 is an endothelial glycoprotein which supports the adhesion of lymphocytes to endothelial cells and mediate lymphocyte re-circulation in a L-selectin-dependent manner [24,25]. VAP-1 has been shown to support sialic acid-dependent adhesion under shear stress and to mediate tethering to the tumour endothelium in human heptacellular carcinoma of T cells [26]. Its cell surface expression is induced under inflammatory conditions, and this may provide an explanation for the observations that SSAO activity is altered under such conditions (see Refs. [1,2,27]). In order to investigate the possible sites of interaction between SSAO and the cell surface, we have investigated the effects of amino sugars, which are known to be involved in cell to cell recognition, on the enzymatic activity of SSAO.

2. Materials and methods

Amine oxidase from bovine plasma was obtained from Sigma Chemical Company along with the other chemicals used. Amino sugars were prepared in distilled water.

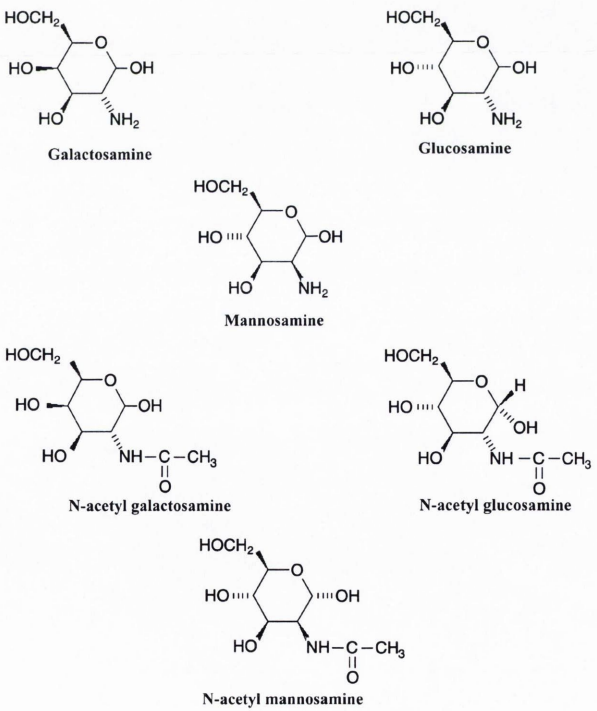


Fig. 1. Chemical structures of the amino sugars used in this study.

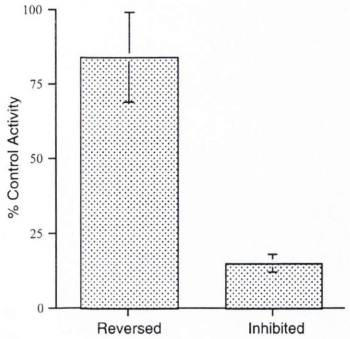


Fig. 2. Reversibility of the inhibition of plasma SSAO by galactosamine.

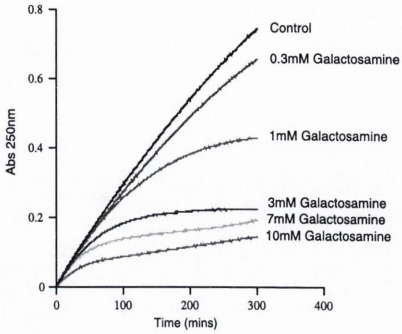


Fig. 3. Time-courses of the oxidation of benzylamine by plasma SSAO in the presence of different concentrations of the galactosamine. Experimental details are given in the text. The results shown are direct output from a single set of directly captured progress curves and are representative of at least six replicates.

All enzyme assays were performed at 37 °C. SSAO activity towards benzylamine was assayed directly by a modification [28] of the method of Tabor et al. [29]. The reaction mixture contained 50 mM potassium phosphate buffer, pH 7.2, and SSAO (concentrations ranging from 12 to 24 $\mu g \cdot ml^{-1}$) in a final volume of 1 ml. The reaction was initiated by the addition of the substrate benzylamine (5 mM) and the rate of change of absorbance was monitored at 250 nm in a Cary 300-Bio spectrophotometer (Varian). The molar extinction coefficient of benzaldehyde at 250 nm was taken to be 13,800 $M^{-1}\ cm^{-1}$ (see Ref. [28]).

Reversibility of inhibition was assessed by dilution experiments. The enzyme (bovine plasma amine oxidase) from Sigma, at a concentration of 1.2 mg protein$\cdot ml^{-1}$, was incubated with 10 mM galactosamine in the presence of 5 mM benzylamine until inhibition was essentially complete, at approximately 2 h. Aliquots of 10 μl were then removed and diluted into the standard benzylamine assay mixture, to give a final protein concentration of 12 $\mu g \cdot ml^{-1}$, for activity determinations.

Kinetic analysis of the time-dependent inhibition of SSAO by galactosamine in the reaction was achieved by fitting the reaction–progress curves data in the presence of the amine substrate according to the equation (see Ref. [30]):

$$[P_t] = [P_\infty](1 - e^{-k't}) \tag{1}$$

where $[P_t]$ and $[P_\infty]$ are the product concentrations at any time, t, and at complete cessation of the reaction, respectively, and k' is the apparent first-order rate constant for the decline in enzyme activity.

The substrate-concentration dependence of the apparent first-order rate constants at each amino sugar concentration was fitted to the relationship,

$$k' = \frac{k_{+3}}{1 + \frac{K_i}{[I]}\left(1 + \frac{[S]}{K_m}\right)} \tag{2}$$

which describes the situation where substrate and inhibitor both compete for non-covalent binding to the enzyme, according to the system:

$$\begin{array}{ccccc} E & \underset{k_{-1}}{\overset{k_{+1}.I}{\rightleftharpoons}} & E.I & \xrightarrow{k_{+3}} & E\text{-}I \\ k_{-2} \Big\uparrow \Big\downarrow k_{+2}.S & & & & \\ E.S & & \xrightarrow{k_{cat}} & E + Products & \end{array} \tag{3}$$

in which K_m is the Michaelis constant in the absence of inhibitor ($\{k_{-2} + k_{cat}\}/k_{+2}$) and, assuming $k_{+3} \ll k_{-1}$ [30], $K_I = k_{-1}/k_{+1}$. Square brackets are used to denote concentrations.

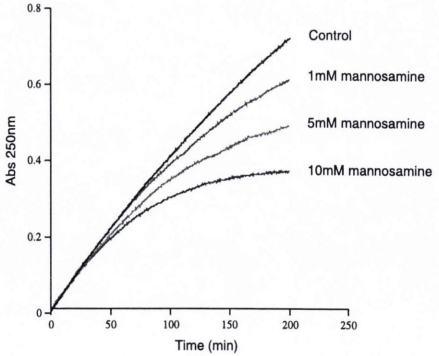


Fig. 4. Time-courses of the oxidation of benzylamine by plasma SSAO in the presence of different concentrations of the mannosamine. The results shown are direct outputs from a single set of directly captured progress curves and are representative of at least six replicates.

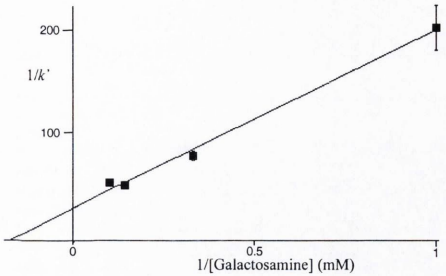


Fig. 5. The variation of the apparent first-order rate constant for time-dependent inhibition of plasma SSAO with the galactosamine concentration, presented for illustrative purposes, as a double-reciprocal plot.

K_I, k_{app} and k_{+3} values were determined using the commercial software programme MacCurveFit 1.5. (Kevin Raner Software, Australia). Data are displayed as a double-reciprocal representation of Eq. (2) for illustrative purposes only.

3. Results

Time-dependent inhibition was observed when SSAO deamination of benzylamine was performed in the presence of amino sugars. Galactosamine, mannosamine and glucosamine (Fig. 1), each at a concentration of 1 mM, all showed the ability to inhibit the plasma SSAO, as well as the activity of tissue-bound SSAO from bovine lung, both in crude microsomal fractions and purified form, prepared as previously described [23]. The parent sugars and their *N*-acetyl derivatives had no effect on the activity of SSAO under these conditions. Reversibility was determined by dilution experiments where the initial rate activity of SSAO returned to control levels after substantial inhibition had occurred by incubation with 10 mM galactosamine for 2 h in the presence of 5 mM benzylamine (Fig. 2).

A range of galactosamine and mannosamine concentrations (up to 25 mM) inhibited SSAO in a concentration- and time-dependent manner (Figs. 3 and 4). The K_i for the galactosamine inhibition was calculated by fitting the reaction progress curves to Eqs. (1) and (2) was found to be 3.9 ± 1.6 mM. The apparent first-order rate (k_{+3}) constant for the time-dependent inhibition was 0.029 ± 0.004 min^{-1} (see Fig. 5). Determination of hydrogen peroxide formation [28] when SSAO was incubated with the amino sugars for periods of up to 2 h indicated that they were not substrates for the enzyme.

4. Discussion

The amino sugars were found to be effective time dependent inhibitors of SSAO, unlike the *N*-acetyl derivative and parent hexoses. This ability and the different potencies of inhibition by the amino sugars suggest that the inhibition shows specificity for the structure of the amino sugar.

The data were also fitted to the equation

$$k' = \frac{k_{app}}{1 + \frac{[S]}{K_m}} \tag{4}$$

which describes the situation where the inhibitor does not form an initial non-covalent complex with the enzyme

$$\begin{array}{ccc} E & \xrightarrow{k.I} & E\text{-}I \\ {\scriptstyle k_{-1}}\uparrow\downarrow{\scriptstyle k_{+1}.S} & & \\ E.S & \xrightarrow{k_{cat}} & E + \text{Products} \end{array} \tag{5}$$

where the *pseudo* first-order $k_{app} = k$ [I], was also attempted for comparative purposes. Such a mechanism would result in a linear dependence of the apparent first-order rate constant on the concentration of amino sugar, or a double-reciprocal plot that passes through the origin (1/rate constant) = 0 when 1/[amino sugar] = 0, which was clearly not the case (see Fig. 5).

Since the kinetics of the time-dependent inhibition follow Eqs. (2) and (3), rather than Eqs. (4) and (5), this would suggest that the amino sugar forms a weak non-covalent complex with SSAO that is then, more slowly transformed to a more tightly bound, but still reversible complex. The possibility that this interaction between SSAO/VAP-1 and amino sugars may serve as a model system for interactions with cell-surface glyco-conjugates that are involved in the adhesion process will be a subject for further studies.

Acknowledgements

We are grateful for support from the Health Research Board, the Irish Society of Toxicology and the EC COST and Socrates programmes.

References

[1] R. Lewinsohn, Mammalian monoamine-oxidizing enzymes, with special references to benzylamine oxidase in human tissues, Braz. J. Med. Biol. Res. 17 (1984) 223–256.
[2] B.A. Callingham, A.E. Crosbie, B.A. Rous, Some aspects of the pathophysiology of semicarbazide-sensitive amine oxidase enzymes, Prog. Brain Res. 106 (1995) 305–321.
[3] G.A. Lyles, Mammalian plasma and tissue-bound semicarbazide-sensitive amine oxidases: biochemical, pharmacological and toxicological aspects, Int. J. Biochem. Cell Biol. 28 (1996) 259–274.
[4] G. Houen, Mammalian Cu-containing amine oxidases(CAOs): new methods of analysis, structural relationships, and possible functions, APMIS. Acta Pathol. Microbiol. Immunol. Scand., Suppl. 96 (1999) 1–46.

[5] S.M. Janes, J.P. Klinman, Isolation of 2,4,5-trihydroxyphenylalanine quinone (topa quinone) from copper amine oxidases, Methods Enzymol. 258 (1995) 20–34.
[6] F. Boomsma, J. van Dijk, U.M. Bhaggoe, A.M. Bouhuizen, A.H. van denMeiracker, Variation in semicarbazide-sensitive amine oxidase activity in plasma and tissue of mammals, Comp. Biochem. Physiol., Part C Pharmacol. Toxicol. Endocrinol. 126 (2000) 69–78.
[7] M. Moldes, B. Fève, J. Pairault, Molecular cloning of a major mRNA species in murine 3T3 adipocyte lineage. Differentiation-dependent expression, regulation and identification as semicarbazide-sensitive amine oxidase, J. Biol. Chem. 274 (1999) 9515–9523.
[8] G. Enrique-Tarancon, I. Castan, N. Morin, L. Marti, A. Abella, M. Camps, R. Casamitjana, M. Palacin, X. Testar, E. Degerman, C. Carpéné, A. Zorzano, Substrates of semicarbazide-sensitive amine oxidase co-operate with vanadate to stimulate tyrosine phosphorylation of insulin-receptor-substrate proteins, phosphoinositide 3-kinase activity and GLUT4 translocation in adipose cells, Biochem. J. 350 (2000) 171–180.
[9] Y. Deng, P.H. Yu, Assessment of the deamination of aminoacetone, an endogenous substrate for semicarbazide-sensitive amine oxidase, Anal. Biochem. 270 (1999) 97–102.
[10] P.H. Yu, Deamination of methylamine angiopathy, toxicity of formaldehyde, oxidative stress and relevance to protein glycoxidation in diabetes, J. Neural Transm., Suppl. 52 (1998) 201–216.
[11] S.N. Young, B.A. Davis, S. Gauthier, Precursors and metabolites of phenylethylaime, *m*- and *p*-tyramine and tryptamine in human lumbar and cisternal cerebrospinal fluid, J. Neurol. Neurosurg. Psychiatry 45 (1982) 633–639.
[12] J.M. Lizcano, D. Balsa, K.F. Tipton, M. Unzeta, The oxidation of dopamine by the semicarbazide-sensitive amine oxidase (SSAO) from rat vas deferens, Biochem. Pharmacol. 41 (1991) 1107–1110.
[13] K.F. Tipton, M. Strolin Benedetti, Amine oxidases and the metabolism of xenobiotics, in: C. Ioannides (Ed.), Enzyme Systems that Metabolise Drugs and Other Xenobiotics, Wiley, Chichester, 2001, pp. 95–146.
[14] N. Morin, J.-M. Lizcano, E. Fontana, L. Marti, F. Smih, P. Rouet, D. Prévot, A. Zorzano, M. Unzeta, C. Carpéné, Semicarbazide-sensitive amine oxidase substrates stimulate glucose transport and inhibit lipolysis in human adipocytes, J. Pharmacol. Exp. Ther. 297 (2001) 563–572.
[15] K. El Hadri, M. Moldes, N. Mercier, M. Andreani, J. Pairault, B. Fève, Semicarbazide-sensitive amine oxidase in vascular smooth muscle cells: differentiation-dependent expression and role in glucose uptake, Vasc. Biol. 22 (2002) 89–94.
[16] N. Mercier, M. Moldes, E.L. Hadri, B. Fève, Semicarbazide-sensitive amine oxidase activation promotes adipose conversion of 3T3-L1 cells, Biochem. J. 358 (Pt 2) (2001) 335–342.
[17] P. Pietrangeli, S. Nocera, P. Fattibene, X. Wang, B. Mondovì, L. Morpurgo, Modulation of bovine serum amine oxidase activity by hydrogen peroxide, Biochem. Biophys. Res. Commun. 267 (2000) 174–178.
[18] Y. Lee, E. Shepard, J. Smith, D.M. Dooley, L.M. Sayre, Catalytic turnover of substrate benzylamine by the quinone-dependent plasma amine oxidase leads to H_2O_2-dependent inactivation: evidence for generation of a cofactor-derived benzoxazole, Biochemistry 40 (2001) 822–829.
[19] X.T. Wang, P. Pietrangeli, M. Mateescu, B. Mondovì, Extended substrate specificity of serum amine oxidase: possible involvement in protein posttranslational modification, Biochem. Biophys. Res. Commun. 223 (1996) 91–97.
[20] M.A. Mateescu, M.J. Dumoulin, X.T. Wang, R. Nadeau, B. Mondov, A new physiological role of copper oxidases: cardioprotection against reactive oxygen intermediates, J. Physiol. Pharmacol. 48 suppl. (2) (1997) 110–121.
[21] L. Wu, M.A. Mateescu, X.T. Wang, B. Mondovì, R. Wang, Modulation of K^+ channel currents by serum amine oxidase in neurons, Biochem. Biophys. Res. Comm. 220 (1996) 47–52.
[22] W.F. Novotny, O. Chassande, M. Baker, M. Lazdunski, P. Barbry, Diamine oxidase is the amiloride-binding protein and is inhibited by amiloride analogues, J. Biol. Chem. 269 (1994) 9921–9925.
[23] J.M. Lizcano, K.F. Tipton, M. Unzeta, Purification and characterization of membrane-bound semicarbazide-sensitive amine oxidase (SSAO) from bovine lung, Biochem. J. 331 (1998) 69–78.
[24] D.J. Smith, M. Salmi, P. Bono, J. Hellman, T. Leu, S. Jalkanen, Cloning of vascular adhesion protein 1 reveals a novel multifunctional adhesion molecule, J. Exp. Med. 188 (1998) 17–27.
[25] S. Jalkanen, M. Salmi, Cell surface monoamine oxidases: enzymes in search of a function, EMBO J. 20 (2001) 3893–3901.
[26] K.F. Yoong, G. McNab, S.G. Hübscher, D.H. Adams, Vascular adhesion protein-1 and I-CAM-1 support the adhesion of tumor-infiltrating lymphocytes to tumor endothelium in human hepatocellular carcinoma, J. Immunol. 160 (1998) 3978–3988.
[27] R. Lewinsohn, Human serum amine oxidase. Enzyme activity in severely burnt patients with cancer, Clin. Chim. Acta 81 (1977) 247–256.
[28] K.F. Tipton, G. Davey, M. Motherway, Monoamine oxidase assays, in: G.P. Taylor, J.P. Sullivan (Eds.), Current Protocols in Pharmacology, Wiley, New York, 2000, pp. 3.6.1–3.6.42.
[29] C.W. Tabor, H. Tabor, S.M. Rosenthal, Purification of amine oxidase from beef plasma, J. Biol. Chem. 208 (1954) 645–661.
[30] K.F. Tipton, Patterns of enzyme inhibition, in: P.C. Engel (Ed.), Enzymology LabFax, Bios Scientific Publishers, Oxford, 1996, pp. 115–174.

The Behaviour of Enzymes in Cells

Keith Tipton Festschrift (an Independent Meeting) held at Trinity College Dublin, 14 September 2002, to celebrate Keith Tipton's First 25 Years in the Biochemistry Department, Trinity College Dublin. Edited by T. Mantle (Trinity College Dublin). Supported by Schering-Plough and by the Biochemical Society and Portland Press.

It can be a complicated life being an enzyme

K.F. Tipton[1], M.I. O'Sullivan, G.P. Davey and J. O'Sullivan

Department of Biochemistry, Trinity College, Dublin 2, Ireland

Abstract

It is becoming increasingly apparent that many well-known enzymes have alternative non-enzymic functions. Similarly, several proteins that were identified as having non-catalytic functions were subsequently found to have enzyme activities. Some examples are considered to illustrate the diversity of alternative functions. The semicarbazide-sensitive amine oxidase (EC 1.4.3.6) is considered in more depth as an example. It was originally believed to be a detoxifying enzyme, but the reaction products may have important signalling functions. Furthermore, this enzyme, from some sources, also behaves as a vascular-adhesion protein. Finally, the challenges posed by such multiplicity of functions for the interpretation of genetic deletion, *in vivo* inhibition and the development of functional protein databases are briefly considered.

Introduction

Most of us working in what used to be called academia are now well used to having to do several different tasks, often simultaneously. Some of these tasks have little to do with the research and teaching that we were appointed to do. It is a relief to know that others are in the same position. In particular, it is becoming increasingly apparent that many of the proteins that we know as enzymes have radically different alternative functions. Perhaps we should not be surprised about this, since we have known for very many years that every time we looked at something it was through the courtesy of glycolytic enzymes forming lens crystallin. However, the number of enzymes known to have spare-time jobs has increased dramatically in recent years.

Table 1 shows a few such enzymes (see [1–13]), chosen to illustrate the diversity of alternative functions. Much more space would be required for a comprehensive list. The list also excludes those enzymes that appear to catalyse widely different reactions, such as the multicatalytic Ku protein (e.g. see [14]), maleylacetoacetate isomerase (EC 5.2.1.2), which is also a glutathione-S-transferase (EC 2.5.1.18) [15], and the *Haemophilus influenzae* NadR protein, which contains both nicotinamide-nucleotide adenylytransferase (E.C 2.7.7.1) and ribosylnicotinamide kinase (EC 2.7.1.22) activities [16]. In some cases, it was an enzyme that was found to have an alter ego, whereas in others a protein whose function was believed to be well established was found to be moonlighting as an enzyme. This multitasking poses a number of challenges, which will be discussed later, but first we will discuss a specific example of an overworked enzyme, which initially seemed to be particularly uninteresting, the so-called semicarbazide-sensitive amine oxidase (SSAO).

What is SSAO and where is it?

'Semicarbazide-sensitive amine oxidase' was a name coined to distinguish a group of enzymes catalysing the oxidative deamination of some aliphatic and aromatic primary amines from the mitochondrial monoamine oxidases [MAOs; with the systematic name of amine:oxygen oxidoreductase (deaminating) (flavin-containing); EC 1.4.3.4]. Both classes of enzyme catalyse the same overall reaction:

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + NH_3 + H_2O_2$$

The substrate specificities of these two enzymes overlap, although the MAOs are also able to oxidize some secondary and tertiary amines, whereas SSAO is not.

The experimental distinction between the two enzymes is often made by their inhibitor sensitivities. The MAOs, which are flavoproteins, are inhibited by some propargylamine derivatives, including clorgyline, deprenyl and pargyline, which are mechanism-based inhibitors that react covalently with the enzyme-bound FAD (see [17]). In contrast, SSAO is insensitive to inhibition by such compounds. Indeed, the even less helpful name of clorgyline-resistant amine oxidase ('CRAO') can be found in some of the earlier literature. Since

Key words: enzyme kinetics, oxidases, oxidation, semicarbazide-sensitive amine oxidase.

Abbreviations used: SSAO, semicarbazide-sensitive amine oxidase; MAO, monoamine oxidase; TOPA, 2,4,5-trihydroxyphenylalanine; 5-HT, 5-hydroxytryptamine; VAP-1, vascular-adhesion protein 1.

[1]To whom correspondence should be addressed (e-mail ktipton@tcd.ie).

©2003 Biochemical Society

Table 1 | Some enzymes with part-time jobs

Enzyme	EC number	Other function(s)	Reference
Aconitase (cytoplasmic)	4.2.1.3	Iron binding and response-element regulation	[1]
Carbonic anhydrase	4.2.1.1	Intestinal salt transport	[2]
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.13	RNA binding, apoptosis	[3]
Some glycolytic enzymes	-	Lens crystallin	[4,5]
Glutathione S-transferase	2.5.1.18	Ligandin	[6]
Glucose-6-phosphate isomerase	5.3.1.9	Major self-antigen in rheumatoid arthritis	[7]
Aldehyde dehydrogenase & transketolase	1.2.1.3 and 2.2.1.1	Major corneal protein ('corneal crystallin')	[7]
Acetylcholinesterase	3.1.1.7	Receptor modulation	[9]
Dipeptidyl peptidase IV	3.4.14.5	CD26 adhesion protein	[10]
5'-Nucleosidase	3.1.3.5	CD73 adhesion protein	[11]
Peptidyl glycine mono-oxygenase	1.14.17.3	Growth hormone	[12]
SSAO	1.4.3.6	VAP-1, etc.	This paper
Ketol-acid reductoisomerase (Yeast I1v5p)	1.1.1.86	Mitochondrial DNA stabilizer of nucleoid parser	[13]

the MAOs are not inhibited by semicarbazide and some other carbonyl-group reagents, it seemed like a good idea at the time to coin the name SSAO in order to distinguish it from MAO. Earlier work on SSAO has been reviewed extensively [18–20].

The systematic name for SSAO is amine:oxygen oxidoreductase (deaminating) (copper-containing); EC 1.4.3.6, a group which also contains some other, quite distinct enzymes. Unfortunately, several other amine oxidases are also inhibited by semicarbazide. These include diamine oxidase, which is also classified in EC 1.4.3.6, but differs from SSAO in having a high activity towards histamine. Lysyl oxidase (protein-lysine 6-oxidase; EC 1.4.3.13) [21,22], and the flavoprotein polyamine oxidase [*N*^{1}-acetylspermidine:oxygen oxidoreductase (deaminating), E.C. 1.5.3.11] [23] are also inhibited by semicarbazide. This adds to the excitement of often not knowing which enzyme is being referred to as SSAO. However, by convention, SSAO is generally used only for the members of the enzymes within EC 1.4.3.6 that are not good at catalysing histamine oxidation.

SSAO is present in plants, animals and microbes. Most mammalian species contain a soluble form of SSAO in blood plasma plus a membrane-bound form of the enzyme that has a relatively high activity in cardiovascular tissue, the lung and adipocytes. It is mainly located on the plasma membrane, and it is believed that the plasma enzyme arises from proteolytic cleavage of the membrane-bound SSAO. The cardiovascular location of the enzyme might suggest that it is involved, together with plasma SSAO, in the removal of potentially toxic amines from the circulation.

Why is SSAO at all interesting?

For many years, SSAO appeared to be a particularly boring enzyme. Its best substrate was found to be benzylamine, which is also a substrate for MAO, and the name 'benzylamine oxidase' was sometimes used. Clearly, to name an enzyme after a substrate that is not known to occur in mammalian tissues is some indication of a lack of faith in its physiological relevance.

Table 2 | Altered levels of SSAO in some disease states

All data refer to the human plasma enzyme, unless otherwise indicated. ↑, increase in plasma SSAO; ↓, decrease in SSAO.

Condition	Plasma SSAO	Reference
Alzheimer's disease	↑ (cerebral blood vessels)	[27]
Burns	↓	[18]
Cancer (solid tumour)	↓	[18]
Cancer (breast)	↓ (rat, chemically induced)	[18]
Cardiac disease	↑	[28]
Congestive heart failure	↑ (increases with severity)	[29]
Diabetes (Types 1 and 2)	↑ (human, rat and sheep)	[18]
Diabetes	↑ (rat plasma and kidney)	[18]
Diabetes	Unchanged (rat aorta, lung and pancreas)	[18]
Diabetic retinopathy	↑↑	[30]
Diabetic atherosclerosis	↑↑	[31]
Hypertension	Unchanged	[18]
Pre-eclampsia	Unchanged (placenta)	[32]
Stroke	No change	[33]

There was a flurry of interest when the carbonyl-containing cofactor that was originally thought to be pyridoxal phosphate was reported to be pyroquinoline quinone ('PQQ'), and this was subsequently shown to be TOPA (2,4,5-trihydroxyphenylalanine; also called 6-hydroxydopa) quinone (see [24]). The enzyme contains 1 mol of copper per active site, which appears to be essential for the conversion of the tyrosine residue in the polypeptide chain of SSAO into TOPA cofactor [25]. The copper does not appear to have any redox role in amine oxidation, and may simply act as a suitably placed positive charge in the active site [26]. In view of what we know of the effectiveness of most copper oxidases, this might appear to be a strange design fault.

The observation that the activity of SSAO appeared to be altered in a number of disease states, as summarized in

©2003 Biochemical Society

Table 2 [18,27–33], did suggest that the enzyme must do something physiologically interesting. Indeed, it appears that elevated SSAO may be a prognostic marker for heart disease and diabetic complications, rather than simply a reflection of the disease state. However, it is only recently that some suggestions as to the significance of such observations have begun to emerge.

What SSAO might do as an enzyme

Many of the substrates for SSAO, including 2-phenylethylamine, tyramine and dopamine, are better substrates for the MAOs. Generally, it would appear that SSAO plays only a small role in metabolizing substrates that are also oxidized by MAO, although it could be more important when MAO is inhibited, or in the rare genetic condition where the MAO genes are deleted [34]. Although 5-hydroxytryptamine (5-HT; serotonin) is not a substrate for SSAO from most sources, it is a good substrate for the enzyme from pig and human dental pulp (see [35]). The enzyme may also play a role in the metabolism of some xenobiotics, such as mescaline and allylamine (see [36]).

Of the physiological amines that are not MAO substrates, aminoacetone and methylamine have been most studied. Methylamine is produced in several catabolic reactions, including the breakdown of adrenaline, which is catalysed by MAO, sarcosine and creatine. It is also a common component of some foods and beverages, and is an atmospheric pollutant. It has been suggested that the high levels of SSAO in the lung are to protect against inhaled methylamine and other volatile amines [37].

Aminoacetone, which may be formed as a result of threonine or glycine metabolism, is oxidized by SSAO to form methylglyoxal, which is cytotoxic and mutagenic. Although there are other pathways of aminoacetone breakdown, SSAO does make a significant contribution to the process [38].

What the products of the SSAO-catalysed reaction might do

SSAO, in common with MAO, catalyses a reaction in which each of the products is potentially nasty. Hydrogen peroxide can act as a source of reactive-oxygen-containing radicals, ammonia is known to be toxic in a number of systems, and the aldehyde products formed may also be toxic. The products common to the oxidation of all amines (ammonia and hydrogen peroxide) are, of course, also important metabolic intermediates in a number of different reactions and, before wildly speculating about the toxicity of the SSAO-catalysed reaction products, it would be necessary to show the enzyme to be capable of producing, or significantly contributing to, toxic levels of these compounds under some conditions. They also have other metabolic and, perhaps, signalling functions. However, since there are many metabolic sources of ammonia and hydrogen peroxide, it necessary to know whether there is something special about the localization or regulation of SSAO that makes its metabolites likely to be particularly important in such responses. Unfortunately, except in the case of glucose transport (discussed below), there is little evidence for this.

It has been suggested that one of the roles of SSAO is the formation of H_2O_2 for the regulation of the function of the glucose transporters GLUT1 and GLUT4 [39,40]. The hydrogen peroxide formed stimulates the transport of the glucose transporters to the cell surface, and thus promotes glucose transport. This is not a specific property of SSAO, since the externally added H_2O_2 and that formed by the MAO-catalysed reaction act similarly. However, a proportion of SSAO in adipocytes has been shown to be associated with the intracellular vesicles containing the GLUT4 glucose transporter, which may suggest a more specific function for this enzyme.

The potential toxicity of some aldehyde products is well documented. The cardiotoxicity of the xenobiotic allylamine has been shown to result from its oxidation to acrolein by SSAO [41]. Formaldehyde, which is the product of methylamine oxidation, can produce irreversible adducts with proteins and single-stranded DNA, among other harmful cross-linkage reactions (see [42]). It might be expected to be oxidized rapidly to formate by the glutathione-dependent formaldehyde dehydrogenase (EC 1.2.1.1). However, that enzyme is not present in the blood plasma, so any formaldehyde produced there would have to be transported into cells, such as the erythrocytes, for metabolism. This may be significant with regard to formaldehyde-induced toxicity in blood vessels [43]. Indeed, the toxicity *in vivo* of methylamine in the rat was shown to be decreased by the inhibition of SSAO [43].

The consumption of relatively large amounts of creatine as a nutritional supplement, in attempts to enhance sports performance, would result in increased methylamine formation, and it has been suggested that the formation of formaldehyde and H_2O_2 from its oxidation might underlie some of the deleterious effects of long-term creatine consumption [44].

Aminoacetone oxidation by SSAO produces methylglyoxal, which is highly cytotoxic. Since the levels of methylglyoxal, as well as those of SSAO activity, are raised in diabetes, this had led to the suggestion that this might contribute to some of the diabetic complications, such as advanced-glycation end product ('AGE') formation [38].

What else might SSAO do?

SSAO appears to be a particularly busy enzyme. There was some interest when it was reported that SSAO was an 'amiloride-binding protein'. Amiloride and some other imidazoline and guanidine compounds are inhibitors of sodium-transporting systems, and therefore are useful as diuretic and hypertensive agents. Investigations of their behaviour revealed the presence of tissue proteins that bound these drugs with some specificity. It now appears that SSAO

©2003 Biochemical Society

may function in this way [45]. MAO is also an amiloride-binding protein, but the plasma and endothelial-surface location of SSAO may make it more important. The enzyme from different sources differ considerably in their binding abilities, and SSAO from ox lung has, for example, been shown to have a low affinity [37]. The possible significance of this binding, which inhibits SSAO activity, for those taking such drugs remains unclear.

Recently, an adhesion protein, vascular-adhesion protein 1 (VAP-1), was found to have sequence identity with SSAO at the cDNA level [46], and was subsequently found to possess SSAO activity. VAP-1 is an endothelial glycoprotein that supports the adhesion of lymphocytes to endothelial cells and mediates lymphocyte recirculation in an L-selectin-dependent manner (see [47] for a review). VAP-1 has been shown to support sialic-acid-dependent adhesion under shear stress, and to mediate tethering of T-cells to the tumour endothelium in human hepatocellular carcinoma [48]. Its cell-surface expression is induced under inflammatory conditions, and this may provide an explanation for the observations that SSAO activity increases under such conditions (see Table 2) [49]. This might appear to be a totally unrelated function of SSAO, although our studies on the interaction of SSAO with amino sugars suggest the H_2O_2 generated in the enzyme-catalysed reaction to be necessary for adhesion [50]. VAP-1 type adhesion is, perhaps not surprisingly, found only in SSAO from some tissues. Since, with the exception of retinal SSAO [51], there appears to be only a single gene for SSAO (see [52]), this suggests some further complexities in the processing of SSAO/VAP-1.

As a final complexity, SSAO appears to be necessary for adipocyte maturation [53,54] and in extracellular matrix development and maintenance, and specifically in the development of normal elastin in vascular smooth muscle [55]. In the case of adipocyte maturation, H_2O_2 formed as a result of substrate oxidation by SSAO appeared to be necessary but, intriguingly, that resulting from MAO-catalysed substrate oxidation was ineffective [53].

Some implications of multitasking

The complexities in the functions of what, at first, seems to be a protein of only minor interest provide an indication of the challenges in determining its quantitative roles in the different processes and under different physiological conditions. The suggestion that SSAO has been put there by God so that you can have complications if you are diabetic appears to underlay some of the literature on this enzyme. Indeed, similar ideas have been commonly expressed for other proteins. Clearly, the effects of inhibition may be far more diverse than might be concluded from a narrow focus on its substrates.

Effects of enzyme modulation

Knock-out experiments are already more difficult to interpret than was thought to be the case as judged from several earlier experiments that did not appreciate the possibilities of redundancy, or that an enzyme may have different functions during development than it does in the adult. An example of this is the A isoenzyme of MAO (EC 1.4.3.4; MAO-A). The human phenotype resulting from the loss of the gene for this enzyme is characterized by compulsive aggression [56], whereas inhibition of the enzyme in the adult is anti-depressive, but without the extreme symptoms seen with the 'knock-outs'. Similarly MAO-A knock-out mice behave aggressively [57], whereas essentially complete inhibition of the enzyme in the adult has much less marked effects. These differences may be attributable to 5-HT, a substrate for this enzyme, having a key role in brain development. Interestingly, maintenance of 5-HT levels is also important for the development of the whisker barrel in the rat [58], but apparently not in its maintenance in the adult.

If these developmental aspects are viewed in the light of multiple protein and, in the case of enzymes, substrate/product functions, knock-out experiments might be expected to result in a more complex, and interesting, phenotype than the simple loss of a single function. As mentioned above, there are also implications for the development and use of enzyme inhibitors for therapeutic purposes.

Classification

Attempts to provide functional classifications for proteins have been fewer than those determined by sequence, superfamily or parent DNA sequences. The well-established IUBMB enzyme nomenclature and classification list [59] classifies enzymes according to the reaction catalysed. It is parsimonious in that, if a number of quite different proteins catalyse the same reaction, such as yeast and mammalian alcohol dehydrogenase, they have the same classification (EC) number. Similarly, isoenzymes are treated as a single entity. Only if there is a significant difference in the reactions catalysed are different EC numbers used (see [60]). This procedure made sense when it was introduced, because nothing was known about the structures of the proteins involved. Despite its limitations, it still forms the basis for enzyme identification in a large number of databases. In the rather few cases where an enzyme was known to have another, unrelated, function, this might be referred to in a comment. For example, the entry for NADPH:quinone reductase (EC 1.6.5.5) has the comment, 'In some mammals the enzyme is abundant in the lens of the eye, where it is identified with the protein ζ-crystallin'.

This will be hardly sufficient in the future. Linkage of each enzyme, perhaps by way of a sequence database, to one of the many separate databases that contains some classification based on other functions might be a way forward. However, this will not be entirely straightforward. In the case of SSAO, for example, not all SSAOs within an organism are VAPs. Whereas the enzyme associated with the vascular endothelium functions in this way, that associated with adipocytes does not. So far, there is no evidence for primary structural differences between these two proteins. It is possible that the adipocyte enzyme is associated with

©2003 Biochemical Society

adhesion to something else, but there are no data to support such an hypothesis. So, at the present state of the art, the binding protein that can be classified as VAP-1 is only a part of the enzyme group known as SSAO.

We are grateful to the Health Research Board for financial support. Thanks are also owed to many people, most of whom are cited in the reference list, for stimulating and convivial discussions.

References

1 Toth, I. and Bridges, K.R. (1995) J. Biol. Chem. **270**, 19540–19544
2 Charney, A.N., Alexander-Chacko, J., Gummaconda, R. and Egnor, R.W. (2002) Biochim. Biophys. Acta **1573**, 141–148
3 Sirover, M.A. (1999) Biochim. Biophys. Acta **1432**, 159–184
4 Farnsworth, P.N., Schleich, T.W., Groth-Vasselli, B. and Kuckel, C.L. (1988) Ophthalmic Res. **20**, 126–131
5 Wistow, G.J., Lietman, T., Williams, L.A., Stapel, S.O., de Jong, W.W., Horwitz, J. and Piatigorsky, J. (1988) J. Cell Biol. **107**, 2729–2736
6 Sheehan, D. and Mantle, T.J. (1984) Biochem. J. **152**, 893–897
7 Matsumoto, I., Staub, A., Benoist, C. and Mathis, D. (1999) Science **286**, 1732–1735
8 Piatigorsky, J. (2000) J. Ocul. Pharmacol. Ther. **16**, 173–180
9 Day, T. and Greenfield, S.A. (2002) Neuroscience **111**, 649–656
10 Aytac, U., Claret, F.X., Ho, L., Sato, K., Ohnuma, K., Mills, G.B., Cabanillas, F., Morimoto, C. and Dang, N.H. (2001) Cancer Res. **61**, 7204–7210
11 Resta, R., Yamashita, Y. and Thompson, L.F. (1998) Immunol. Rev. **161**, 95–109
12 Downey, E. and Donlon, J. (1997) Arch. Biochem. Biophys. **345**, 193–198
13 Bateman, J.M., Perlman, P.S. and Butow, R.A. (2002) Genetics **161**, 1043–1052
14 Stronati, L., Gensabella, G., Lamberti, C., Barattini, P., Frasca, D., Tanzarella, C., Giacobini, S., Toscano, M.G., Santacroce, C. and Danesi, D.T. (2001) Cancer **92**, 2484–2492
15 Blackburn, A.C., Woollatt, E., Sutherland, G.R. and Board, P.G. (1998) Cytogenet. Cell Genet. **83**, 109–114
16 Singh, S.K., Kurnasov, O.V., Chen, B., Robinson., H., Grishin, N.V., Osterman, A.L. and Zhang, H. (2002) J. Biol. Chem. **277**, 33291–33299
17 Tipton, K.F., Davey, G. and Motherway, M. (2000) in Current Protocols in Pharmacology (Taylor, G.P. and Sullivan, J.P., eds), pp. 3.6.1–3.6.42, John Wiley, New York
18 Lyles, G.A. (1996) Int. J. Biochem. Cell Biol. **28**, 259–274
19 Callingham, B.A., Crosbie, A.E. and Rous, B.A. (1995) Prog. Brain Res. **106**, 305–321
20 Houen, G. (1999) APMIS, Suppl. **96**, 1–46
21 Smith-Mungo, L.I. and Kagan, H.M. (1998) Matrix Biol. **16**, 387–398
22 Wang, S.X., Nakamura, N., Mure, M., Klinman, J.P. and Sanders-Loehr, J. (1997) J. Biol. Chem. **272**, 28841–28844
23 Kunimoto, S., Nosaka, C., Xu, C.Z. and Takeuchi, T. (1989) J. Antibiotics (Tokyo) **42**, 116–122
24 Klinman, J.P. and Mu, D. (1994) Annu. Rev. Biochem, **63**, 299–344
25 Cai, D., Williams, N.K. and Klinman, J.P. (1997) J. Biol. Chem. **272**, 19277–19281
26 McGuirl, M.A. and Dooley, D.M. (1999) Curr. Opin. Chem. Biol. **3**, 138–144
27 Ferrer, I., Lizcano, J.M., Hernandez, M. and Unzeta, M. (2002) Neurosci. Lett. **321**, 21–24
28 Boomsma, F., de Kam, P.J., Tjeerdsma, G., van den Meiracker, A.H. and van Veldhuisen, D.J. (2000) Eur. Heart J. **21**, 1859–1863
29 Boomsma, F., van Veldhuisen, D.J., de Kam, P.J., Man in't Veld, A.J., Mosterd, A., Lie, K.I. and Schalekamp, M.A.D.H. (1997) Cardiovasc. Res. **33**, 387–391
30 Gronvall-Nordquist, J.L., Backlund, L.B., Garpenstrand, H., Ekblom, J., Landin, B., Yu, P.H., Oreland, L. and Rosenqvist, U. (2001) J. Diabetes Complications **15**, 250–256
31 Karádi, I., Mészáros, Z., Csányi, A., Szombathy, T., Hosszúfalusi, L., Romics, L. and Magyar, K. (2002) Clin. Chim. Acta **323**, 139–146
32 Sikkema, J.M., Franx, A., Fijnheer, R., Nikkels, P.G., Bruinse, H.W. and Boomsma, F. (2002) Clin. Chim. Acta **324**, 31–38
33 Garpenstrand, H., Ekblom, J., von Arbin, M., Oreland, L. and Murray, V. (1999) Eur. Neurol. **41**, 20–23
34 Fitzgerald, D.H. and Tipton, K.F. (2002) J. Neural Transm. **109**, 251–265
35 O'Sullivan, M., Tipton, K.F. and McDevitt, W.E. (2002) Arch. Oral Biol. **47**, 399–406
36 Tipton, K.F. and Strolin Benedetti, M. (2001) In Enzyme Systems that Metabolise Drugs and Other Xenobiotics (Ioannides, C., ed), pp. 95–146, John Wiley & Sons, Chichester
37 Lizcano, J.M., Tipton, K.F. and Unzeta, M. (1998) Biochem. J. **331**, 69–78
38 Mathys, K.C., Ponnampalam, S.N., Padival, S. and Nagaraj, R.H. (2002) Biochem. Biophys. Res. Commun. **297**, 863–869
39 Enrique-Tarancon, G., Castan, I., Morin, N., Marti, L., Abella, A., Camps, M., Casamitjana, R., Palacin, M., Testar, X., Degerman, E. et al. (2000) Biochem. J. **350**, 171–180
40 El Hadri, K., Moldes, M., Mercier, N., Andreani, M., Pairault, J. and Feve, B. (2002) Arterioscler. Thromb. Vasc. Biol. **22**, 89–94
41 Conklin, D.J., Trent, M.B. and Boor, P.J. (1999) Toxicology **138**, 137–154
42 Yu, P.H. (1998) J. Neural Transm. **52** (suppl), 201–216
43 Deng, Y., Boomsma, F. and Yu, P.H. (1998) Life Sci. **63**, 2049–2058
44 Yu, P.H. and Deng, Y. (2000) Med. Hypotheses **54**, 726–728
45 Novotny, W.F., Chassande, O., Baker, M., Lazdunski, M. and Barbry, P. (1994) J. Biol. Chem. **269**, 9921–9925
46 Smith, D.J., Salmi, M., Bono, P., Hellman, L., Leu, T. and Jalkenen, S. (1998) J. Exp. Med. **188**, 17–27
47 Tohka, S., Laukkanen, M.-L., Jalkanen, S. and Salmi, M. (2001) FASEB J. **15**, 373–382
48 Yoong, K.F., McNab, G., Hübscher, S.G. and Adams, D.H. (1998) J. Immunol. **160**, 3978–3988
49 Kurkijarvi, R., Yegutkin, G.G., Gunson, B.K., Jalkanen, S., Salmi, M. and Adams, D.H. (2000) Gastroenterology **119**, 1096–1103
50 O'Sullivan, J., O'Sullivan, M., Tipton, K.F., Unzeta, M., Hernandez, M. del M. and Davey, G. (2003) Biochim. Biophys. Acta **1647**, 367–371
51 Imamura, Y., Noda, S., Mashima, Y., Kudoh, J. and Oguchi, Y. (1998) Genomics **51**, 293–298
52 Zhang, X. and McIntire, W.S. (1996) Gene **179**, 279–286
53 Mercier, N., Moldes, M., El Hadri, K. and Feve, B. (2001) Biochem. J. **358**, 335–342
54 Fontana, E., Boucher, J., Marti, L., Lizcano, J.M., Testar, X., Zorzano, A. and Carpene, C. (2001) Biochem. J. **356**, 769–777
55 Langford, S.D., Trent, M.B. and Boor, P.J. (2002) Cardiovasc. Toxicol. **2**, 141–150
56 Brunner, H.G., Nelen, M., Breakefield, X.O., Ropers, H.H. and van Oost, B.A. (1993) Science **262**, 578–580
57 Shih, J.C. and Chen, K. (1999) Neurobiology **7**, 235–246
58 Rebsam, A., Seif, I. and Gaspar, P. (2002) J. Neurosci. **22**, 8541–8552
59 http://www.chem.qmw.ac.uk/iubmb/enzyme/
60 Boyce, S. and Tipton, K.F. (2000) Bioinformatics **16**, 34–40

Received 17 March 2003

©2003 Biochemical Society

Monoamine Oxidases: Certainties and Uncertainties

Keith F. Tipton*, Sinead Boyce, Jeff O'Sullivan, Gavin P. Davey and Joe Healy

Department of Biochemistry, Trinity College, Dublin 2, Ireland

Abstract: A great deal has been learned about the behaviour of monoamine oxidase in the 75 years since it was first discovered, but there is still a great deal left to understand. This review concentrates on the dynamic aspects of our knowledge of the interactions of MAO with substrates and inhibitors and how it may collaborate with other enzymes, with particular emphasis on aspects that remain to be clarified.

INTRODUCTION

Other articles in this volume will deal with specific aspects of monoamine oxidase structure, behaviour and perceived function. The purpose of this chapter is to provide a general introduction to the background of such work, in terms of the possible functions of MAO, and to highlight some aspects where further work is necessary or where our basic assumptions may not be as firmly based as often supposed.

Our knowledge of the metabolism of amines in mammalian systems has a long history. Some of the early history is summarised, in terms of notable anniversaries, in Table **1**. Clearly, the arbitrary restriction to quinquennia means that there is much important work that is missing, and reference to some of the earlier reviews on monoamine oxidase [1-5] will fill in any gaps.

REACTION CATALYSED

Monoamine oxidase (monoamine oxygen oxidoreductase (deaminating)(flavin-containing); EC 1.4.3.4; MAO) oxidises primary aliphatic and aromatic amines as well as some secondary and tertiary amines according to the overall reaction

$$RCH_2NR'R'' + O_2 + H_2O \longrightarrow RCHO + NR'R'' + H_2O_2$$

Kinetic studies have shown the reaction to involve the binding of the amine substrate to the enzyme before oxygen. The reaction proceeds in two steps. In the first of these reduction of the enzyme-bound FAD results in the formation of the aldehyde product and ammonia, whereas the second step entails the reoxidation of the enzyme-bound FAD by O_2 with the formation of hydrogen peroxide.

$$RCH_2NH_2 + MAO \xrightarrow[+H_2O]{-2H} RCHO + NH_3 + Rreduced\text{-}MAO$$

$$reduced\text{-}MAO + O_2 \longrightarrow MAO + H_2O_2$$

With primary amines the first partial reaction is believed to proceed *via* an imine intermediate, which is then hydrolysed by water to the final product.

$$RCH_2NH_2 \xrightarrow{-2H} RCH{=}NH \xrightarrow{+H_2O} RCHO + NH_3$$

However, hydrolysis of this type of intermediate does not occur in the case of some irreversible inhibitors or with the neurotoxin MPTP [see, e.g., 6].

The release of the aldehyde and/or ammonia before the binding of oxygen would be consistent with the double-displacement, or ping-pong, mechanism (see Fig. **1**) that kinetic studies have shown to be followed by several substrates. However, the precise reaction mechanism followed will depend upon the relative rates of product (ammonia and aldehyde) release compared to the rate of oxygen binding and this has been shown to depend upon the assay conditions, the substrate used and, possibly, the enzyme source or preparation [see 7-10]. In terms of (Fig. **1**), if $k_{+4} >> k_{+8}[O_2]$ the double-displacement reaction will operate, whereas if $k_{+4} << k_{+8}[O_2]$ the reaction will be sequential, i.e., involving a kinetically significant ternary (E.Aldehyde.Oxygen or E.Ammonia.Oxygen) or quaternary (E.Aldehyde.Ammonia.Oxygen) complex.

Interest in the kinetic mechanism followed by the enzyme is not merely esoteric, since it affects how the enzyme will operate within the cell. The location of MAO on the mitochondrial outer membrane, where it may be in competition with other oxygen consumers, such as cytochrome oxidase, might suggest that local competition for oxygen could be important, since MAO has a high K_m for oxygen with many of its substrates. However, a feature of the double-displacement mechanism is that decreasing the concentration of one substrate lowers both the apparent maximum velocity ($V^{app.} = \{1 + K_m^B/[B]\}/V$) and K_m ($K_m^{Aapp.} = K_m^A/\{1 + K_m^B/[B]\}$) towards the other [see, e.g., 11]. This contrasts with sequential mechanisms, where the dependence of the apparent maximum velocity is the same but the behaviour of the apparent K_m ($K_m^{Aapp.} = \{K_m^A + K_s^A K_m^B/[B]\}/\{1 + K_m^B/[B]\}$) is more complex and will depend upon the apparent dissociation constant for the first substrate to bind ($K_s^A = k_{-1}/k_{+1}$ in the sequential rate equation Fig. **1**). Thus, at low amine concentrations the activity will be effectively buffered against fluctuations in the oxygen concentration. In contrast the sequential mechanism can be much more sensitive to fluctuations in the concentrations of its substrates. This is illustrated for the situation where $K_s^A = K_m^B$ in (Fig. **2**). The precise dependence of a sequential mechanism will depend on the

*Address correspondence to this author at the Department of Biochemistry, Trinity College, Dublin 2, Ireland; E-mail: ktipton@tcd.ie

0929-8673/04 $45.00+.00 © 2004 Bentham Science Publishers Ltd.

relative values of $K_s^A = K_m^B$, if $K_s^A > K_m^B$ the sensitivity towards substrate fluctuations will be even more marked, whereas it will be less so when $K_s^A < K_m^B$, tending to the double-displacement situation as K_s^A tends to zero.

Table 1. MAO: Some historical Anniversaries: 2003

120 YEARS:
∑ Minkowski O (1883) Uber Spaltungen im Thierkorper. *Arch. Exp. Pathol. Pharmakol.* 17, 445-465.
75 YEARS:
∑ Hare MLC (1928) Tyramine oxidase. I. A new enzyme system in liver. *Biochem. J.* 22, 968 979.
65 YEARS:
∑ Zeller EA.(1938) Uber den enzymatishcen Abbau von Histamin und Diaminen. *Helv. Chim Acta* 21, 881-890.
45 YEARS:
∑ Kline NS (1958) Clinical experiences with iproniazid (Marsilid). *J. Clin. Exptl. Psychopathol. Quart. Rev. Psychiat. Neurol.* 19, Suppl. !, 72-78.
35 YEARS:
∑ Johnston JP (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* 17, 1285-1297.
20 YEARS:
∑ Cawthon RM & Breakefield XO. (1983) Differences in the structures of monoamine oxidases A and B in rat clonal cell lines. Biochem Pharmacol. 32, 441-418.
∑ Langston JW, Ballard P, Tetrud JW. & Irwin, I. (1983). Chronic parkinsonism in humans due to a product or meperidine-analogue synthesis. *Science* 219, 979-980.
15 YEARS
∑ Bach AW, Lan NC, Johnson DL, Abell CW, Bembenek ME, Kwan SW, Seeburg PH & Shih JC (1988) cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proc Natl Acad Sci USA* 85,4934-4938.
∑ Hsu YP, Weyler W, Chen S, Sims KB, Rinehart WB, Utterback MC, Powell JF & Breakefield XO (1988) Structural features of human monoamine oxidase A elucidated from cDNA and peptide sequences.*J Neurochem* 1988 51, 1321-1324.
10 YEARS
∑ Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA (1993). Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. Science 262, 578-580.

The rest is modern history

Such considerations suggest that the kinetic mechanism followed by MAO may be advantageous within the cellular compartment where it is located. It should also be noted that, as will be discussed later, knowledge of the kinetic mechanism followed by the enzyme is advantageous for the design of effective inhibitors.

INHIBITORS AS DRUGS

The plethora of existing MAO inhibitors have been subject to many reviews and the question as to whether we need more has often been raised [see 12], and then promptly ignored. The trivial answer is that the existing ones are now out of patent and, therefore, it is necessary to design ones and claim that they are better. However, as additional information on the behaviour of the enzymes becomes available, it may be appropriate to design more effective inhibitors. This may become desirable as new functions of MAO inhibitors are found. However, some of the apparently desirable properties of MAO inhibitors appear to be less than clear-cut. For example, their neuroprotective, or 'neuro-rescuing' actions appear to be independent of MAO inhibition [for examples see 13-15]. It remains unclear whether the dopamine-sparing effects of such compounds, such as (-)-deprenyl, confer additional benefits in the treatment of Parkinson's disease [see 16]. The radioprotective effects of some MAO inhibitors also appear to be independent of their abilities to inhibit the enzyme [17, 18]. The reported beneficial effects of (-)-deprenyl in Alzheimer's disease have recently been questioned [19].

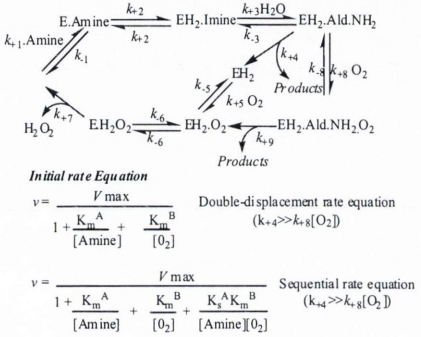


Fig. (1). Alternative kinetic mechanisms followed by monoamine oxidase for the oxidative deamination of a primary amine. The products released in the steps governed by k_{+4} and k_{+9} are aldehyde (Ald) and ammonia.

The major disadvantages of the use of inhibitors of MAO-A as antidepressants were hepatotoxicity and the potentiation of tyramine (the "cheese reaction"). The first of these problems, appears to have been an adverse effect of drugs, such as barbiturates, which used often to be co-administered with the hydrazine MAO inhibitors to aid sleep and appears to be no longer a difficulty since the discontinuation of barbiturates. The development of reversible and selective MAO-A inhibitors also appears to have abolished the problem of the cheese reaction [for reviews see 5, 20]. This led to the argument that selectivity towards MAO-A was not necessary a feature for reversible and competitive MAO inhibitors. Since the cheese reaction is a result of the inhibition of peripheral, particularly intestinal, MAO-A [see 21], a brain selective MAO inhibitor would be expected to be safe in this respect, as, for example, has recently been shown to be the case with the non-selective irreversible inhibitor *N*-propargyl-(3R)-aminoindan-5-yl-ethyl methylcarbamate hemitartate (TV3326) [22].

Such considerations raise the question as to whether a reversible or an irreversible inhibitor is more appropriate. In comparing reversible and irreversible inhibitors, it must be remembered that the rate of turnover, and hence recovery

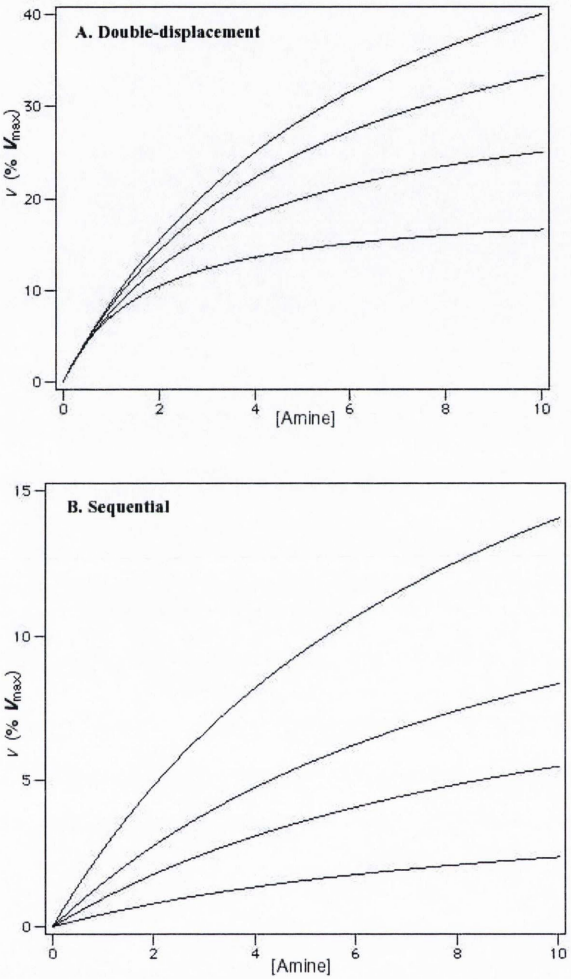


Fig. (2). Effects of reaction mechanism on the sensitivities of an enzyme to fluctuations in the concentrations of its substrates. The curves were constructed from the equations for the double-displacement (**A**) and the sequential (**B**) mechanisms shown in Fig.1. The fixed conditions were $K_m^A = K_m^B = K_s^A = 10$. The varied substrate was, in terms of Fig. 1, the amine and the second substrate (oxygen) was held at the following fixed multiples of its K_m value, in ascending order of the curves: $[O_2] = 0.5\ K_m^B$, $[O_2] = 1.0\ K_m^B$, $[O_2] = 2.0\ K_m^B$, and $[O_2] = 4.0\ K_m^B$. Comparison between the two panels indicates the insensitivity of the double-displacement system at low amine concentrations to fluctuations in the oxygen concentration, as compared with the behaviour of the sequential system under these set conditions.

from irreversible inhibition is relatively slow and differs considerably between tissues and species (see Fig. **3**). The main differences between reversible and irreversible inhibitors are shown in Table **2** (for further details see 11, 23]. Tight-binding inhibitors, which include harmaline [24], Ro 41-1049 and lazabemide [25. 26], generally behave as reversible inhibitors, although their high affinities may result in their dissociating from the enzyme rather more slowly.

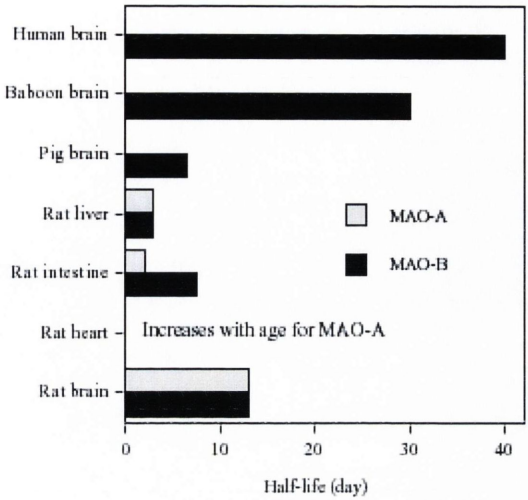


Fig. (3). The turnover rates of MAO-A and –B from different species and tissues. The values shown would correspond to the half-times for the reappearance of MAO following irreversible inhibition.

In the case of reversible inhibitors, the inhibition may be competitive, mixed, non-competitive or uncompetitive in type (see Fig. **4**). Only in the case of competitive inhibitors can increasing substrate concentrations fully displace inhibitor from the enzyme. It is this feature that provides the safety factor against the effects of tyramine ingestion. The behaviour of the different types of reversible inhibitor in response to rising substrate concentrations is illustrated in (Fig. **5**). Since inhibition of an enzyme will result in a rise in the levels of its endogenous substrate(s), this will tend to reduce the effect of a competitive inhibitor. Such compounds are often administered at very high concentrations, relative to the K_I value to minimize that effect, which obviously increases the risk of side effects as a result of non-specific interactions and the production of unwanted metabolites.

Because competitive inhibitors frequently resemble the products of an enzyme-catalysed reaction it has been a relatively simple matter to design such inhibitors by synthesising non-reactive substrate analogues. The conditions for competitive inhibition are that the enzyme can either bind substrate or inhibitor; it cannot bind both at once. This is often interpreted as meaning that the inhibitor binds to the active site of the enzyme. This is not the only possibility, however, and it is also possible for the inhibitor to bind to a site that is distinct from the active site and cause a shape change in the enzyme that prevents substrate binding. The converse, that substrate-binding should also cause a shape change that prevents inhibitor binding is also required for simple competitive inhibition. Much of our thinking about enzyme- substrate and enzyme-inhibitor interactions was based on the assumption that the active site would be readily accessible. The earlier crystallographic studies appeared to bear this out. However, it has become increasingly evident that for many enzymes, including MAO and the semicarbazide-sensitive amine oxidases (see later), the substrate has to diffuse through an entrance cavity or passage before reaching the active site. This suggests that a compound may inhibit by blocking the entrance to the active site. Such a possibility of substrate and inhibitor preventing each-others access, may underlie the puzzling behaviour of some inhibitors, including, as will be discussed later, the imidazolines. It also indicates that caution is needed in assessing the results of docking and modelling experiments that are based on oversimplified models of the enzyme.

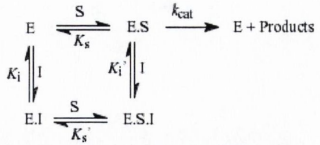
As can be seen from the diagram in (Fig. **5**), the effect of an uncompetitive inhibitor becomes more pronounced as the substrate concentration increases. Thus such inhibitors should be beneficial if they could be administered at concentrations sufficient to act as "substrate buffers", having little effect at low substrate concentrations, but becoming more powerful if the substrate concentration rises above the normal range. Uncompetitive inhibitors have, however, been less easy to design. The classical view is that they bind only to the enzyme-substrate complex and not to the free enzyme. However, in the double-displacement mechanism, as shown for MAO in (Fig. **1**), any compound that binds solely to the free reduced enzyme (EH_2) will be an uncompetitive inhibitor with respect to the amine substrate (and competitive with respect to oxygen). This could act as an aid to the design of uncompetitive inhibitors if they are required. However, it should be noted that if the compound bound both to the free enzyme and EH_2, the inhibition would be mixed. Since mixed inhibitors behave as a combination of competitive and uncompetitive inhibition, their effects with respect to substrate concentration will lie between these two extremes, the exact behaviour depending on the relative values of the dissociation constants for the E.I (K_I) and E.S.I (K_I') complexes.

Table 2. Conopeptides being Developed for the Treatment of Neurological Conditions

Name	Conopeptide	*Conus* Species (*)	Target	Stage	Company **	Comment	Reference
ACV1	_-Conotoxin Vc1.1	*Conus victoriae (m)*	Competitive blocker of selected *neuronal-type* nicotinic ACh receptors	Preclinical	Metabolic Pharmaceuticals Ltd, Melbourne, Vic, Australia	Effective against peripheral neuropathic pain in animal models and accelerates functional recovery of injured neurons	[1, 94]
	rho- Conotoxin TIA	*Conus tulipa (p)*	Reversible noncompetitive inhibitor of _-1 adrenergic receptors	Preclinical	Xenome, Ltd., Brisbane, Qld., Australia	Acts as a reversible noncompetitive inhibitor of alpha-1 adrenergic receptors	[72]
AM336	w-conotoxin CVID	*Conus catus (p)*	Blocks N-type calcium channel, Ca((v)2.2) calcium channel variant.	Stage II	AMRAD Corp. - under license from Univ. of Queensland	Being developed for neuropathic pain. Reported to have a better therapeutic index than Prialt™	[25]
SNX-III, C1002, Ziconotide, Prialt™	w-conotoxin MVIIA	*C. magus (p)*	N-type calcium channels	Stage III	Elan Corporation (Elan Pharmaceuticals), CA, USA	Significant pain relief to patients in clinical trials. Side-effects in some patients. Hence call for repeat of Stage III clinical trials for cancer pain. Also trialed (as C1002) for neuro-protection in ischemic stroke and coronary bypass surgery but results were disappointing and trials have been abandoned.	[21, 110] [82, 83, 111,113] [22-24]
Xen2174	c-conopeptides (chi-CTX MrIA/B)	*Conus marmoreus (m)*	Acts as reversible noncompetitive inhibitor of the neuronal noradrenaline transporter	Preclinical (Stage I to begin in 2004)	Xenome, Ltd., Brisbane, Qld., Australia	Being developed to "treat certain types of pain, for which there is currently a lack of effective treatment" neuropathic pain.	[72]
CGX-1160	Contulakin-G	*Conus geographus (p)*	Binds to neurotensin receptor	Stage II	Cognetix Inc, Salt Lake City, USA	Short term management of post-operative pain	[71]
CGX-1007	Conantokin-G	*Conus geographus (p)*	Selective inhibitor of the NMDA receptor (NR2B subtype)	Stage II	Cognetix Inc, Salt Lake City, USA	Potent antinociceptive effects in several models of injury-induced pain. Also, control of seizures in intractable epilepsy	[71]
CGX-	Conantokin-T	*Conus tulipa (p)*	Selective inhibitor of the NMDA receptor (NR2A and NR2B) subtypes	Stage II	Cognetix Inc, Salt Lake City, USA	Potent antinociceptive effects in several models of injury-induced pain	[71]

* Prey preference for *Conus* species: *p = piscivorous* (fish-hunting); *m = molluscivorous* (mollusk hunting); *v = vermivorous* (worm-hunting).
Note: This list does not attempt to be comprehensive. For other examples of *Conus* peptides being investigated for therapeutic potential see the excellent reviews by Heading [21,23].
** Websites of commercial developers: Elan Corporation http://www.elan.com ; Cognetix Inc. http://www.cognetix.com ; Xenome Pty Ltd: http://www.xenome.com ; Amrad Corp. http://www.amrad.com.au ; Univ. of Melbourne: Cone Shell and Conotoxin HomePage http://grimwade.biochem.unimelb.edu.au/cone/ ; Metabolic Pharmaceuticals Ltd.: http://www.metabolic.com.au

Mechanism-based inhibitors provide the most specific type of irreversible inhibitor because the enzyme has not only to recognise and bind the inhibitor to the active site; it also has to perform a part of its normal catalytic function to generate an activated species that then reacts with the enzyme (see Fig. **6**). This means that there are, at least, two factors to be optimized to obtain high selectivity and potency. These are the affinity of the enzyme for non-covalent binding to form the E.I complex and the rates of the subsequent steps leading to the irreversible inhibition [see e.g.,



Inhibition	Relationships	v =	I_{50} =	% inhibition = 100x
Competitive	$K_I' = \bullet$ $K_s' = \bullet$	$\frac{V_{max}[S]}{[S]+K_m'(1+[I]/K_i)}$	$K_i(1+\frac{[S]}{K_m})$	$\frac{[I]}{[I]+K_i(1+[S]/K_m)}$
Uncompetitive	$K_I = \bullet$ $K_s' = \bullet$	$\frac{V_{max}[S]}{[S](1+[I]/K_i')+K_m}$	$K_i'(1+\frac{K_m}{[S]})$	$\frac{[I]}{[I]+K_i'(1+K_m/[S])}$
Mixed*	$K_I \pi K_I'$ $K_s \pi K_s'$	$\frac{V_{max}[S]}{[S](1+[I]/K_i')+K_m(1+[I]/K_i)}$	$\frac{(K_m+[S])}{\frac{K_m}{K_i}+\frac{[S]}{K_i'}}$	$\frac{[I]}{(K_m+[S])+(\frac{K_m}{K_i}+\frac{[S]}{K_i'})[I]}{\frac{K_m}{K_i}+\frac{[S]}{K_i'}}$
Noncompetitive*	$K_I = K_I'$ $K_s = K_s'$	$\frac{V_{max}[S]}{([S]+K_m)(1+[I]/K_i)}$	K_i	$\frac{[I]}{[I]+K_i}$

Notes: *The mixed and noncompetitive inhibition systems only give more complex relationships unless all binding steps occur under conditions of themodynamic equilibrium, where $K_m = K_s$, *or* if $K_s' = \bullet$, when steady state conditions may also obtain.Under steadystate conditions $K_m = k_{cat}K_s$.

Fig. (4). Reversible inhibitor relationships.

27].These two factors should ideally co-operate as has been shown to be the case for the selectivities of clorgyline and (-)-deprenyl, where a higher affinity for binding to the more sensitive form of MAO is further enhanced by a higher reactivity of that form to give the irreversible adduct. However, this is nor always the case for selective inhibitors. In the case of pargyline the, somewhat weaker, selectivity towards MAO-B is solely a result of differences in K_i values for MAO-A and -B, with the rates of the reactions leading to covalent adduct formation being similar with both enzyme forms [28]. With some mechanism-based inhibitors we have shown that the two kinetic processes to have opposing effects in terms of selectivities [see e.g., 29].

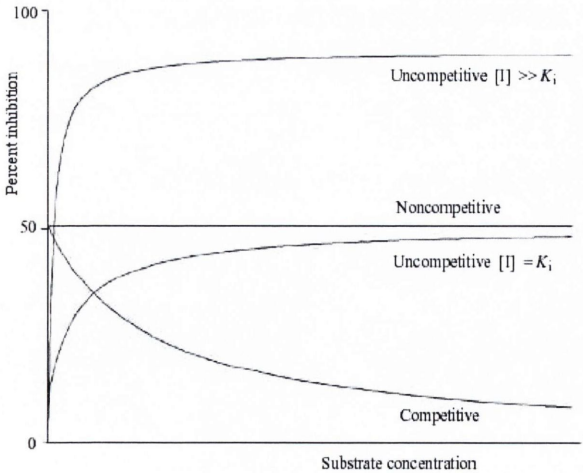


Fig. (5). Effects of substrate concentration on the inhibition given by the different types of reversible inhibitor. In all curves, $K_m = 10$ and $V = 100$, arbitrary units. The inhibitor concentrations and K_I values used were. Competitive inhibition: [I],= 5, $K_i = 5$; uncompetitive inhibition: [I] = 5, $K_i = 5$ ([I] = K_I) and [I] = 100, $K_i = 5$ ([I] >> K_I); noncompetitive inhibition: [I] = K_i = K_I = 5.

A. Simple mechanism-based inhibition

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E.S \xrightarrow{k_{in}} E\text{-}I$$

If the rate of transformation of the non-covalent enzyme.inhibitor complex (E.I) to give the irreversibly inhibited enzyme (E-I), which may occur in more than one step, is slow, relative to the rate of dissociation of E.I back to free enzyme (E) and inhibitor, and the concentration of inhibitor, [I], >> [E]the rate of inhibition (v_i) will given by:

$$v_i = \frac{k_{in}[E][I]}{K_s + [I]} \quad \text{where } K_s = k_{-1}/k_{+1}$$

B. "Suicide substrate"

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E.S \begin{cases} \xrightarrow{k_{in}} E\text{-}I \\ \xrightarrow{k_{cat}} E + P \end{cases}$$

$$\text{Partition ratio } (r) = k_{cat}/k_{in}$$

Fig. (6). Simple kinetics of mechanism-based inhibitors and "suicide substrates".

Since mechanism-based inhibitors behave like substrates, in binding to the active site of the enzyme and being converted to the reactive species through a process resembling the normal catalytic process of the enzyme, it is perhaps not surprising that it is possible for the reactive species to break down to form the product. In such cases the formation of product and the mechanism-based inhibition of the enzyme will be competing reactions according to the general scheme shown in (Fig. **6**). Such inhibitors are sometimes referred to as "suicide substrates". An additional factor must be taken into account with such compounds is the relative effectiveness of the compound as a substrate compared to its effectiveness as an inhibitor. This is defined as the partition ratio, *r*, as shown in (Fig. **6**). Provided the inhibitor is present in sufficient excess to cause complete irreversible inhibition of the enzyme, the ratio of product formed at complete inhibition, [P•], divided by the enzyme concentration, [E], will also give *r*, the number of mol of product formed per mol of enzyme at complete inhibition. The full equations describing the behaviour of such are rather cumbersome [see e.g., 23, 30] but it is possible to determine the other parametres necessary to assess inhibitor effectiveness.

In the case of the inhibition of MAO by clorgyline and (-)-deprenyl there is no detectable formation of products during the inhibitory process [28, 31, 32] and thus they behave as simple mechanism-based inhibitors. However, phenelzine [see 33], MD 780236 (3-(4[(3-chlorophenyl)methoxy]phenyl)-5-[(methylamino)-methyl]-2-oxazolininone methane sulphate) [34], MPTP [35], and milacemide (2-(*n*-pentylamino)acetamide) [36] were all found to act as both substrates and mechanism-based inhibitors of MAO. Clearly, the importance of such behaviour depends on the pharmacological application of the compound being studied. If it is intended as an inhibitor, a high value of *r* will mean that higher amounts will have to be used to inhibit MAO and the products formed may have unwanted side-effects. Alternatively, if the compound is intended as a pro-drug, the inhibition of the enzyme that forms it may limit its value.

DETERMINATION OF THE ISOENZYMES

The two isoenzymes of monoamine oxidase are present in most mammalian tissues (see reviews cited above). These enzymes, MAO-A and -B, were originally distinguished by their sensitivities to inhibition by the acetylenic inhibitors clorgyline and deprenyl and by their substrate specificities. Typically MAO-A is inhibited by low concentrations of clorgyline and catalyses the oxidative deamination of 5-hydroxytryptamine (5-HT), whereas MAO-B is inhibited by low concentrations of (-)-deprenyl and is active towards benzylamine and 2-phenylethylamine (PEA).

These specificities and inhibitor sensitivities have frequently been used in attempts to quantitate the relative amounts of activity MAO-A and -B present different tissues. In physiological terms this has the advantage that it determines the enzymes in terms of the activities present. However, these substrate specificities are not absolute. For example, 5-HT is a substrate for MAO-B as well as MAO-A in rat brain and liver. However, in the rat brain the K_m and V_{max} values for MAO-B are 6-fold higher and 9-fold lower, respectively, than those for MAO-A. Hence, relatively low concentrations of 5-HT will be oxidised essentially by MAO-A alone, whereas at very high concentrations the B form can contribute about 10% of the total activity. Similarly, PEA is a substrate for MAO-A but with a considerably higher K_m and a V_{max} value that is 5-6-fold lower than the corresponding values for MAO-B. Thus, although the A form can contribute to a percentage of the total activity at very high concentrations of PEA, only the activity of the B form is important at low concentrations of this substrate. In rat heart, which contains very little MAO-B, it has been reported to be possible to detect low levels of benzylamine oxidation by MAO-A.

These factors are important in interpreting the results of attempts to determine the relative proportions of the two enzymes in tissues from their activities towards arbitrarily fixed concentrations of such substrates. In most species examined tyramine is a substrate for both enzymes, as are dopamine and noradrenaline in human brain. Although tyramine has been frequently regarded as a substrate for both forms of MAO, the kinetic parametres towards this substrate differ. With the enzymes from rat liver mitochondria, for example, the K_m and V_{max} values of MAO-A towards this substrate were found to be 107 μM and 11 nmol.min^{-1}.mg protein^{-1}, respectively, whereas the values for MAO-B were 579 μM and 20 nmol.min^{-1}.mg protein^{-1}, respectively. Therefore, the proportions of the total activity towards tyramine that are contributed by each of the two forms will depend to some extent on the concentration of substrate. Thus about 32% of the total rat liver MAO activity towards 50 μM tyramine will be due to MAO-B, but this proportion will rise to 57% at a tyramine concentration of 1 mM. Hence, there is no simple subdivision into specific MAO-A, specific MAO-B and mixed substrates but rather a continuum. Such considerations may also affect attempts to determine MAO distributions by histochemical procedures based on staining for H_2O_2 formation from specific substrates [see, e.g., 37-39].

Attempts to quantitate the amounts of the enzymes present based on mRNA levels are likely to give misleading results since MAO is one of the growing numbers of proteins where it is now established that the mRNA levels do not correspond to the amounts of protein expressed [40]. Thus immunological procedures or inhibitor binding are to be preferred for quantitation. Immunological procedures can be robust and sensitive [see 40, 41], but they will give the total concentration of MAO-A and -B protein present, with no information as to whether it is all active.

Since one mol of an irreversible inhibitor should be sufficient to inhibit one mol of enzyme, attempts have been made to titrate tissue preparations with such inhibitors to determine the minimum amount necessary to inhibit the activity completely [42]. However, the procedures have been shown to be inaccurate owing to non-specific binding of the inhibitor [31]. Of the inhibitor-binding procedures, both irreversible [see 32, 43] and tight-binding reversible inhibitors [see 24-26] have been used. The use of radioactively labeled mechanism-based inhibitors, such as clorgyline, deprenyl and pargyline, has the advantage that inhibition depends on a part of the normal catalytic activity of the enzymes and thus would not detect any enzyme molecules that are totally devoid of activity. It has been shown possible to determine the amounts of activity present *in vivo* by use of PET scanning after administration of ^{18}F- or ^{11}C-labelled inhibitors [see 44, 45].

ARE THERE ODDITIES OUT THERE?

We seem to have single genes each, on the X chromosome, for MAO-A and for MAO-B, but these do contain introns, which would give scope for splice variants. There are also possibilities for post-translational modifications leading to heterogeneity. Such modifications, however, do not appear to include glycosylation. It has been known for many years that single base changes in the encoding DNA are responsible for several heredity diseases, whereas other changes may have more subtle effects or no effects at all. The technology for measuring alterations has now become so straightforward that the approach has changed to determining the presence of such single-nucleotide polymorphisms (SNPs) in populations and assessing whether they confer specific disease susceptibility or personality attributes. This aspect will be discussed in relation to personality type elsewhere in this volume. There is no doubt that SNPs may be important in several aspects of disease susceptibility and treatment response [see 46- 50]. This is not an appropriate place to delve into the niceties of risk-factor analysis, but it is worth pointing out that you need very large numbers of samples for any results to be meaningful.

Certainly there is evidence for heterogeneity in the behaviour of MAO. Thus, as discussed below, only a proportion of human liver MAO-B acts as an imidazoline-binding protein, whereas platelet MAO-B is apparently devoid of this property. In our own studies on the inhibition of human brain MAO-B by the milacemide analogue (*S*)-2-[4-(3-flourobenzyl-oxy)-benzylamino] propionamide, which had previously been reported to be a selective MAO-B inhibitor [51], we found it to be a time-dependent inhibitor of the enzyme (see Fig. **7**). However, the MAO-B activity of MAO-B in one of the 6 post-mortem human brain samples was less sensitive to inhibition, with no significant time-dependence (see Fig. **7**). Indeed its inhibitor sensitivities resembled those of MAO-A from this and the other human brain samples. The activity of MAO-B in this sample was comparable with those in the other human brain samples studied and then there was no indication of medication or substance abuse that might have interfered with the behaviour of the enzyme. Furthermore, and the age, cause of death and post-mortem delay was within the range of the other subjects.

It is not normal to publish results with a single sample, but the atypical behaviour of one human brain sample, that

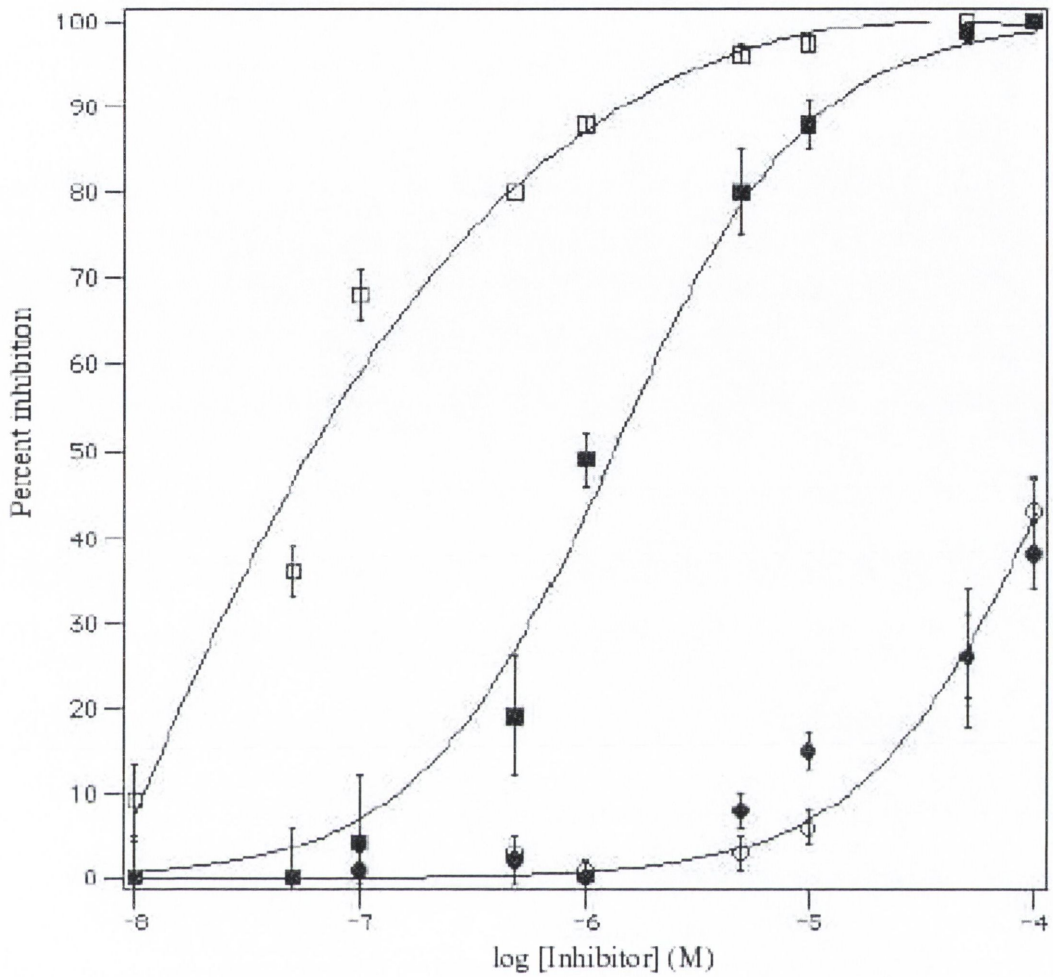


Fig. (7). Inhibition of human brain MAO -B by (*S*)-2-[4-(3-flourobenzyl-oxy)-benzylamino] propionamide. The tissue samples were assayed for MAO-B activities in the presence of the indicated inhibitor concentrations, either without prior enzyme inhibitor preincubation (open symbols) or after incubation for 30 min at 37 C for 30 min (closed symbols) before the addition of substrate (20 mM 2-phenylethylamine). Data are expressed as percentage inhibition with respect to controls carried through the same procedure in the absence of inhibitor. ® & n, MAO-B (representative data from 4 separate brain samples); O & n, the "atypical MAO-B sample. Each value is the mean ± s.e.r. from triplicate determinations.

there is a group of individuals with a MAO-B that responds differently. Such behaviour, which is well known with several other enzymes, could have far-reaching consequences for the therapeutic use of MAO inhibitors. Unfortunately, after the assays had revealed this anomalous behaviour, the preparation was unsuitable for studies at the molecular level, is hoped that all those working in this area will be vigilant in looking for other subjects exhibiting atypical MAO.

A complexity that remains to be explained is the report of Szutowicz *et al.* [52] that MAO-A, or a component tightly bound to it, can modify the behaviour of MAO-B. These authors reported that preincubation of platelet MAO-B with purified human placental MAO-A resulted in a modification of the MAO-B to yield a form of the enzyme with both MAO-A and MAO-B substrate and inhibitor affinities whilst retaining MAO-B immunospecificity. To our knowledge, this phenomenon has not been further investigated in detail.

ENDOGENOUS INHIBITORS

There have been several reports of endogenous inhibitors of MAO, but some of these compounds involved remain unidentified. There have been several reports of an inhibitor in CSF [53, 54] and brain [55, 56] and a peptide, neurocatin, has been reported to inhibit MAO-A in intact, but not disrupted, synaptosomes [57, 58]. Low molecular weight materials which may act as endogenous MAO inhibitors include isoquinolium derivatives [59], harman [60], phosphatidylserine [61] and quinolinic acid [62]. However, it is not certain whether these compounds reach sufficient concentrations *in vivo* for inhibition to be significant.

Several groups have reported platelet MAO levels to be reduced in schizophrenic subjects [see 63 - 66]. However, others have failed to find such a correlation [see 67 - 69]. It is possible that failure to take adequate account of the effects

of medication may account for some of these discrepancies; since Fang *et al.* [70] have shown haloperidol and its metabolites inhibit MAO. Furthermore, cigarette smoking, which has been shown to inhibit MAO [71], may be a further source of error unless taken into account. However, even when this is taken into account, the lowered platelet MAO levels were found to persist in some studies. Thus it might be tempting to ascribe these discrepancies to diagnostic or methodological differences. The presence of a dissociable MAO inhibitor might result in the activities being dependent, to some extent, on sample treatment. Indeed, there have been persistent reports of an endogenous inhibitor of platelet MAO-B that is elevated in schizophrenics [63, 72 - 74; but see Wise [75].

Perhaps the most studied endogenous MAO inhibitor is tribulin [for reviews see 76, 77]. It was first identified in human urine but has since been shown to be widely distributed in mammalian tissues and body fluids. Indeed, some of the unidentified MAO inhibitors discussed above may correspond to tribulin components. Tribulin has been shown to be a complex mixture that includes selective inhibitors of MAO-A (tribulin A), MAO-B (tribulin B), central and peripheral nenzodiazepine receptors (BZc and BZp), respectively. Isatin appears to be the major MAO-B inhibitory component of tribulin but the nature of tribulin A is less clear. MAO-A inhibitory components isolated from human urinary tribulin were found to include esters of tryptamine and tyramine metabolites, but there appear to be species differences in the compositions of tribulin A [78, 79]. Human urinary tribulin levels have been shown to be increased under conditions of stress and anxiety and decreased under sedation, although the levels of tribulin A and B may not change in concert [see 80]. Furthermore, the levels of tribulin in rat brain appear to parallel the urinary levels and changes in the levels of biogenic amines and their metabolites are consistent with tribulin affecting MAO activities *in vivo* [see 81-83].

Studies on the pharmacological behaviour of the amiloride and some other imidazoline and guanidine compounds, which are inhibitors of sodium-transporting systems used as diuretic and hypertensive agents, revealed the presence of tissue proteins, "amiloride- binding proteins or imidazoline I_2 sites" [for review see 84], which bound these drugs quite specifically. MAO [85] may function in this way. There appear to be dramatic differences in the abilities of MAO from different tissues to bind these compounds. For example, MAO-A from human liver and placenta have been shown to bind imidazolines in a manner that is prevented by irreversible inhibitors, such as clorgyline. Similarly, the binding of imidazolines to MAO-B from human liver is prevented by pargyline and deprenyl. However, human platelet MAO-B has been shown to bind only very weakly [86]. Furthermore, it appears that only about 10% of human liver MAO-B is capable of binding imidazolines [87]. Gene knock-out experiments in mice have shown that the binding sites for idazoxan were unaffected by the deletion of MAO-A but completely abolished in MAO-B knock-out animals [88]. This complete abolition may represent a species difference, since in the rat brain and liver it appears that there are additional non-adrenergic binding sites, with the monoamine oxidases contributing only about 60 % of the total [89].

The drug binding is reversible but it appears that the imidazoline binding sites on both MAO-A and -B are distinct from the active sites [see 90]. This may explain why the effects of imidazolines on MAO activity appears to depend on the specific imidazoline used and, perhaps the enzyme source. For example, idazoxan has been reported to bind to rat liver MAO but not to inhibit its enzyme activity, whereas some other imidazolines were inhibitors [see 91]. The physiological significance of this binding for those taking such drugs remains unclear.

THE OPPOSITION

MAO is not the only enzyme that can catalyse the oxidation of monoamines. The so-called semicarbazide-sensitive amine oxidases (amine:oxygen oxidoreductase (deaminating) (copper-containing) EC 1.4.3.6; SSAO) as well as the copper-binding protein ceruloplasmin and some monooxygenases are also active in this respect.

SSAO

The properties and behaviour of these enzymes have recently been reviewed in some detail [92-95]. This term covers a rather diverse group of copper-containing enzymes, which also appear to differ significantly between tissues and species. Their sensitivity to inhibition by carbonyl-group reagents, such as semicarbazide, is a result of the presence of a 6-hydroxydopa; 2,4,5-trihydroxyphenylalanine (TOPA) quinone residue as the redox cofactor. This distinguishes it from the monoamine oxidases, which are not inhibited by semicarbazide. Conversely SSAO, at least in some species, is insensitive to inhibition by the acetylenic monoamine oxidase inhibitors, such as clorgyline. Because of this SSAO has sometimes been referred to as clorgyline-resistant amine oxidase (CRAO).

In most mammals the enzyme exists in tissue-bound and soluble (plasma) forms, but there are wide species and tissue differences in the activities. Homology-modelling studies suggest that tissue-bound enzyme contains a short intracellular domain, a single trans-membrane domain and a long extracellular domain that includes the catalytic site. Plasma SSAO appears to arise from proteolytic cleavage of membrane-bound SSAO and it has been suggested that both the liver and bone tissue may contribute.

SSAO is present, in varying amounts in many mammalian tissues. Relatively high activities of SSAO are associated cardiovascular smooth muscle cells, adipose tissue, lung and cartilage. It is absent from the nerves and glial cells of the brain, but present in the microvessels of brain and, thus may contribute to the 'blood-brain barrier'. Although absent from nerves in the CNS, it appears that the enzyme may be associated with the nerves in dental pulp [96]. There is a distinct form of SSAO in retina, which is encoded by a separate gene [97], whereas a single gene appears to be responsible for encoding the SSAO in other tissues [98].

There are large species differences in the specificities of SSAO, but the nonphysiological amine benzylamine is a good substrate for the mammalian enzymes. Because of this, plasma SSAO has sometimes been referred to as

benzylamine oxidase. High SSAO activities are found in pig and sheep plasma, the levels are very much lower in human plasma and it is often difficult to detect any at all in the rat. The levels of the tissue-bound enzyme also vary widely between species but do not parallel those of the plasma enzyme.

The physiological substrates are believed to include aminoacetone and methylamine, which are not MAO substrates. SSAO substrates that are also oxidatively deaminated by MAO include 2-phenylethylamine, tyramine, dopamine. Although 5-HT is not a substrate for SSAO from most sources, it is a good substrate for the enzyme from pig and human dental pulp. SSAO also catalyses the oxidative deamination of a number of xenobiotics [see 99], including mescaline and the anti-malarial drug, primaquine. However, differences in substrate specificities between the SSAO enzymes from different mammalian sources complicate any assessment. For example, mescaline has been shown to be oxidised more efficiently than benzylamine by pig plasma SSAO but the human enzyme has no detectable activity towards mescaline.

The three-dimensional structure of mammalian SSAO has yet to be determined. However, resolution of the crystal structures of the copper-containing amine oxidases from several other sources has revealed considerable differences in active-site accessibility that may account for specificity differences. Possible effects of variations in glycosylation, which differ considerably between tissues and species, cannot be excluded.

Ceruloplasmin

Ceruloplasmin (EC 1.16.3.1) is a blue coloured plasma protein, which binds up to 95% of circulating copper. It catalyses the oxidation of Fe^{2+} to Fe^{3+} and is classified as ferroxidase (EC 1.16.3.1). Although copper binding and iron homeostasis may be its main functions [see 100], it also catalyses amine oxidation; mediating a four-electron reduction of oxygen by substrate without releasing O_2^- or H_2O_2 as products or intermediates [101]. It has relatively broad oxidase specificity for organic molecules and has the ability to reduce O_2 to H_2O in successive one-electron steps. Physiologically important substrates include dopamine, 6-hydroxydopamine, noradrenaline, adrenaline and serotonin [102, 103]. It also oxidises aromatic diamines, which have been shown to bind to a distinct site on the enzyme from that occupied by monoamines [104]. Ceruloplasmin is most active at low pH values and shows no significant activity towards benzylamine. The protein is expressed in liver, which is believed to be the origin of circulating ceruloplasmin, and other tissues, with lung being a relatively rich source [see 105].

In the brain the enzyme has been shown to be glycosylphosphatidylinositol-anchored (GPI-anchored) to astrocytes [106], where it appears to be important in controlling cellular iron transport [107]. The protein has also been shown to be associated with neurones and its concentrations have been shown to be elevated, in a brain-region specific manner, in Parkinson's, Huntington's and Alzheimer's diseases [108]. This may be a stress response, since ceruloplasmin is known to be an acute-phase protein in the periphery, where its concentrations in liver and plasma increase in response to a number of stress conditions [109]. However, ceruloplasmin deficiency results in neurodegeneration [see 110, 111]. The physiological significance of the amine oxidase activities of ceruloplasmin remains to be established.

Monooxygenases

The flavoprotein monooxygenase (FMO) and some members of the cytochrome *P-450* family of monooxygenases can catalyse N-hydroxylation of some amines. Although subsequent steps may result in deamination, most interest in these enzymes has been in the field of drug detoxification and the potential carcinogenicity of the N-hydroxides formed [see e.g., 112, 113]. There have been rather few studies on the importance of such oxidative pathways relative to the amine oxidases and in the case of the biogenic amines more attention has been focused on ring oxidation by the *P-450* family [see 114]. Such studies have also been rendered difficult by the fact that some amine oxidase inhibitors also affect cytochrome *P-450*-dependent monooxygenases [see 115]. However, Yu *et al.* [116] have shown that MAO but not the cytochrome *P-450* enzymes is responsible for tryptamine oxidation in human brain and liver.

FMO is a member of the unspecific monooxygenase family of enzymes (EC1.14.14.1). Rather little is known about its activity towards MAO substrates. However, it appears to be responsible for the oxidation of amphetamine, which is not a substrate for either MAO or SSAO.

Of the other xenobiotics studied, MAO, SSAO and the cytochrome *P-450* monoxygenases can catalyse the oxidation of primaquine, but at least in rat liver, MAO appears to play the dominant role in the oxidation of this drug [117]. The selective serotonin reuptake inhibitor citalopram and its demethylated derivatives are substrates for MAO. Members of the cytochrome *P-450* monooxygenase family catalyse the demethylation of citalopram. However, it appears that MAO plays the more important role in citalopram metabolism in human liver [118].

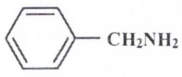
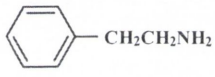
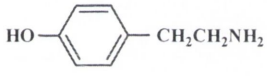
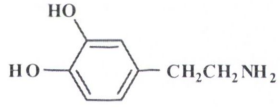
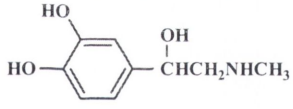
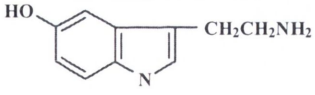
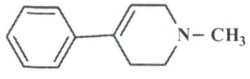
A great deal more work will be necessary to find out the interplay between the monooxygenases and the amine oxidases in the metabolism of different amines. Furthermore, the effects those xenobiotics that are MAO or SSAO substrates on the normal metabolism of endogenous and exogenous amines should be considered.

SUBSTRATE COMPETITION

The large tissue and species and tissue variations in SSAO activity and specificity together with the paucity of our knowledge of the activities of ceruloplasmin and the monooxygenases, makes it difficult to appreciate how these enzymes may compete, or collaborate, in substrate oxidation. Table **3** lists some substrates for MAO, SSAO and ceruloplasmin.

The fact that the active site of the tissue-bound SSAO appears to be located in the extracellular domain would be consistent with its being involved, together with the plasma

Table 3. Amine Oxidase Activities Towards Some Amines

SUBSTRATE	MAO	SSAO	Ceruloplasmin
CH_3NH_2 Methylamine	No	Yes	Unknown
$CH_2{=}CHCH_2NH_2$ Allylamine	No	Yes	Unknown
$CH_3-C(=O)-CH_2NH_2$ Aminoacetone	No	Yes	Unknown
$CH_3(CH_2)_3CH_2NH_2$ *n*-Pentylamine	Yes	Yes	Unknown
 Benzylamine	Yes	Yes	Yes
 2-Phenethylamine	Yes	Yes	Yes
 Tyramine	Yes	Yes	Yes
 Dopamine	Yes	Yes	Yes
 Adrenaline	Yes	No	Yes
 5-Hydroxytryptamine	Yes	Yes (in dental pulp only)	Yes
 *MPTP	Yes	No	No

*MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

enzyme, in the inactivation of potentially toxic amines in the blood. In contrast, the monoamine oxidases are intracellular enzymes, which are associated with the mitochondrial-outer membrane.

The large species and tissue differences in SSAO, discussed above, have led to an acceptance that it is not advisable to attempt to extrapolate from the situation in experimental animals to that in the human. However, there are also species and, perhaps, tissue differences in the behaviour of MAO, which are too often ignored. Species differences occur in the behaviour of MAO towards clorgyline [119], MPTP and its derivatives [120, 121], milacemide and its derivatives [122], tricyclic and other antidepressants [123, 124] and oxadiazolones and

Table 4. Amine oxidases recognized in the IUBMB Enzyme List†

EC Common name	EC number	Other names	Reaction	Comments
amine oxidase (flavin-containing)	1.4.3.4	**monoamine oxidase**; **MAO**; tyramine oxidase; tyraminase; amine oxidase; adrenalin oxidase; epinephrine oxidase; polyamine oxidase; serotonin deaminase; adrenaline oxidase; spermidine oxidase; spermine oxidase. *Systematic:* amine:oxygen oxidoreductase (deaminating) (flavin-containing)	$RCH_2NH_2 + H_2O + O_2 = RCHO + NH_3 + H_2O2$	A flavoprotein (FAD). Acts on primary amines, and usually also on some secondary and tertiary amines.
amine oxidase (copper-containing)	1.4.3.6	**semicarbazide-sensitive amine oxidase; SSAO**; diamine oxidase; diamino oxhydrase; histaminase; amine oxidase; monoamine oxidase; amine oxidase (pyridoxal containing); benzylamine oxidase; histamine deaminase; histamine oxidase; Cu-amine oxidase; *Systematic:* amine:oxygen oxidoreductase (deaminating) (copper-containing)	$RCHNH_2 + H_2O + O_2 = RCHO + NH_3 + H_2O_2$	A group of enzymes including those oxidizing primary monoamines, diamines and histamine. They are copper quinoproteins.
ethanolamine oxidase*	1.4.3.8	*Systematic:* ethanolamine:oxygen oxidoreductase (deaminating)	ethanolamine + $H_2O + O_2$ = glycolaldehyde + NH_3 + H_2O_2	A cobamide-protein.
putrescine oxidase	1.4.3.10	putrescine:oxygen oxidoreductase (deaminating)	putrescine + $H_2O + O_2$ = 4-aminobutanal + NH_3 + H_2O_2	A flavoprotein (FAD). 4-Aminobutanal condenses non-enzymically to 1-pyrroline.
cyclohexylamine oxidase*	1.4.3.12	*Systematic:* cyclohexylamine:oxygen oxidoreductase (deaminating)	cyclohexylamine + H_2O + O_2 = cyclohexanone + NH_3 + H_2O_2	A flavoprotein (FAD). Some other cyclic amines can act instead of cyclohexylamine, but not simple aliphatic and aromatic amides.
protein-lysine 6-oxidase	1.4.3.13	**lysyl oxidase** *Systematic:* protein-L-lysine:oxygen 6-oxidoreductase (deaminating)	peptidyl-L-lysyl-peptide + $H_2O + O_2$ = peptidyl-allysyl-peptide + $NH_3 + H_2O_2$	Also acts on protein 5-hydroxylysine.
polyamine oxidase	1.5.3.11	PAO *Systematic:* N^1-acetylspermidine: oxygen oxidoreductase (deaminating)	N^1-acetylspermine + H_2O + O_2 = N^1-acetylspermidine + 3-aminopropanal + H_2O_2	A flavoprotein requiring Fe^{2+}. Also acts on N^1-acetylspermidine and N^1,N^{12}-diacetylspermine.
hydroxylamine oxidase*	1.7.3.4	hydroxylamine oxidoreductase; HAO *Systematic:* hydroxylamine:oxygen oxidoreductase	hydroxylamine + O_2 = nitrite + H_2O	A hemoprotein with seven *c*-type hemes and one *P*-460-type heme per subunit.

Notes *These enzymes are not known to occur in mammalian systems.

† This list excludes the flavin-containing oxidases which act on free amino acids (D-aspartate oxidase (EC 1.4.3.1); D-amino-acid oxidase (EC 1.4.3.3), glycine oxidase (EC 1.4.3.19), L-glutamate oxidase (EC 1.4.3.11), etc.).

oxadiazolethiones [125]. Species differences are not uncommon for other enzymes, but always appear to surprise each time that it is re-discovered for MAO. Indeed, sequence differences might lead one to suspect that the enzymes from different sources might not behave identically ox liver MAO-B appears to have only 82% identity to the rat enzyme, as deduced from the full-length cDNA clone, and 88% identity the human enzyme.

It has also been shown that there are quite pronounced species differences in the oxidation of noradrenaline and dopamine [126, 127]. Nevertheless, it is still common to read that dopamine is a substrate for MAO-B and noradrenaline is a substrate for MAO-A, whereas noradrenaline has been shown to be a substrate for both enzymes in human and rat brain [128] and, for example, in human arteries [129, 130]. Similarly, dopamine is a substrate for both forms of MAO in human brain [131] and

rat liver [132-134], as well in brain regions from several other species [135].

When attempting to determine the relative contributions of SSAO and MAO to the metabolism of amines in specific tissues, it is important to bear in mind that SSAO is likely to form products extracellularly, whereas MAO does so intracellularly. This may explain why MAO makes a large contribution to the metabolism of tyramine during intestinal transport, whereas SSAO plays no significant role, even under conditions where MAO is inhibited [21]. Similarly, the relatively high SSAO levels in lungs may protect against inhaled methylamine and other volatile amines [see 136]. However, when perfused rabbit lung preparations were studied it was found that MAO played a major role in the presystemic elimination of amines such as 2-phenylethylamine, with SSAO making no significant contribution [137]. Under appropriate experimental conditions, however, SSAO has been shown to work effectively in concert with MAO in some tissue preparations. Thus, the presser effects of tyramine in the perfused rat mesenteric arterial bed, were potentiated if both MAO and SSAO were inhibited but not by inhibition of either enzyme alone [138]. In contrast, SSAO inhibitors alone were found to potentiate the contractile effects of sympathomimetic amines on a rat anococcygeus muscle preparation [139]. The possibility that SSAO may play a more important role under conditions where MAO is inhibited or genetically deficient is suggested by a transient rise in its activity following MAO inhibition *in vivo* [140]. Application of the approaches of "systems biology" may be necessary before the roles of the different amine metabolising enzymes can be fully appreciated.

WHAT'S IN A NAME?

One often feels it necessary to apologise before introducing a subject as unexciting as nomenclature. Although the need for standardised, universally accepted, systems of nomenclature in chemistry, genetics, enzymology, etc. has often been stressed, it is the acceptance of standard names that usually causes the problem. It is rare to find people who will admit that they find nomenclature an interesting subject, but many who profess contempt for it can get very excited if it is suggested that their pet nomenclature should be changed in the interest of clarity or uniformity. Many scientists still argue that precise nomenclature is unnecessary, since they are well aware of the enzyme systems on which they are working [see 141, for a definitive statement of this attitude]. However, when the use of names can result in people doing sensible experiments with the wrong enzyme, it is necessary to attempt to do something.

The definitive list of names and classification numbers (EC numbers) of characterised enzymes is provided in the Enzyme List of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) [142]. The information about each of the enzymes recognised as an amine oxidase, shown in Table **4**, indicates that there are difficulties in the names that are used for these enzymes and ample scope for ambiguity between monoamine oxidase (MAO; EC 1.4.3.4) and semicarbazide-sensitive amine oxidase (SSAO, EC 1.4.3.6). Indeed, there are several publications purporting to be about monoamine oxidase, which actually concern SSAO.

The names that are ascribed by the NC-IUBMB to these two enzymes do not follow the usual rules adopted by that body, which are to name enzymes according to the reaction(s) that they catalyse and not by their cofactor requirements [see143]. The difficulty known is sometimes, which substrate(s) to choose. The reason for this departure from the rules is that EC 1.4.3.4 and EC 1.4.3.6 were originally distinguished as monoamine oxidase (also known as tyramine oxidase or tyraminase) and diamine oxidase (DAO, also known as histaminase) [144]. Those names were changed when it was found that MAO could catalyse the deamination of some diamines, and that DAO from some sources was active towards monoamines but had little activity towards histamine.

The name SSAO is a rich source of confusion, since some other amine oxidases are also inhibited by semicarbazide. These include the enzymes that are diamine oxidases (also classified as EC 1.4.3.6) and lysyl oxidase (protein-lysine 6-oxidase; EC 1.4.3.6). Lysyl oxidase differs from the other members of this group in having lysine tyrosylquinone rather than TOPA-quinone as cofactor. Since the specificities of diamine oxidase (DAO), which is found in high levels in the intestine, kidney, thymus gland and placenta, and SSAO overlap, it is not always clear from publications which enzyme was being studied. By usage, SSAO has become defined by a negative: the semicarbazide-sensitive amine oxidase that has little or no activity towards histamine and diamines such as putrescine, cadaverine, spermidine and spermine, which are DAO substrates. In these terms, SSAO is also a monoamine oxidase and since, as mentioned above, the IUBMB enzyme nomenclature and classification system is a functional system, based on the reaction catalysed, it would be appropriate to attempt to distinguish between these enzymes in terms of their substrates rather than their cofactors. Thus what is now known as SSAO might be called methylamine oxidase, the term diamine oxidase would then be reserved for those enzymes that have a high activity for diamines such as histamine and the classifications of polyamine oxidase and lysyl oxidase would be unchanged (see Table **4**). Semicarbazide would also remain of value in distinguishing DAO from the flavin-containing putrescine oxidase, which it does not inhibit.

A minimum change to the present EC classifications of MAO and SSAO, for discussion and the opprobrium likely when anyone has the temerity to suggest a change to any names, is suggested in Table **5**.

WHY SHOULD SSAO HAVE ALL THE FUN?

It is becoming clear that many enzymes may have functions that are distinct from their established catalytic functions. Thus, although ceruloplasmin can catalyse the oxidation of some amines, it principle functions appear to be copper binding and as a ferroxidase (EC 1.16.3.1). The activities of SSAO appears to be particularly diverse in this context [for reviews see 95, 145], although there appears to be a dependence of some of these other functions and

Table 5. Proposed Modifications to the Nomenclature of Some Amine Oxidases

Present entry **EC 1.4.3.4** **Common name:** amine oxidase (flavin-containing) **Reaction:** $RCH_2NH_2 + H_2O + O_2 = RCHO + NH_3 + H_2O_2$ **Other name(s):**monoamine oxidase; tyramine oxidase; tyraminase; amine oxidase; adrenalin oxidase; epinephrine oxidase; MAO; polyamine oxidase; serotonin deaminase; adrenaline oxidase; spermidine oxidase; spermine oxidase, monoamine:O_2 oxidoreductase (deaminating) **Systematic name:** amine:oxygen oxidoreductase (deaminating) (flavin-containing) Comments: A flavoprotein (FAD). Acts on primary amines, and usually also on some secondary and tertiary amines.
Suggested entry **EC 1.4.3.4** **Common name:** monoamine oxidase **Reaction:** $RCH_2NHR' + H_2O + O_2 = RCHO + R'NH_2 + H_2O_2$ **Other name(s):**monoamine oxidase; tyramine oxidase; tyraminase; amine oxidase; adrenalin oxidase; epinephrine oxidase; MAO; serotonin deaminase; adrenaline oxidase; *polyamine oxidase; spermidine oxidase; spermine oxidase* **Systematic name:** amine:oxygen oxidoreductase (deaminating) (flavin-containing) **Comments:** A flavoprotein (FAD). Acts on primary amines, and also on some secondary and tertiary amines. It differs from EC 1.4.3.6, methylamine oxidase, in that respect and in being inactive towards methylamine.
Present entry **EC 1.4.3.6** **Common name**: amine oxidase (copper-containing) **Reaction:** $RCH_2NH_2 + H_2O + O_2 = RCHO + NH_3 + H_2O_2$ **Other name(s):** diamine oxidase; diamino oxhydrase; histaminase; amine oxidase; monoamine oxidase; amine oxidase (pyridoxal containing); benzylamine oxidase; histamine deaminase; histamine oxidase; Cu-amine oxidase; amine oxygen oxidoreductase; diamine:O_2 oxidoreductase (deaminating); semicarbazide-sensitive amine oxidase; SSAO **Systematic name:** amine:oxygen oxidoreductase (deaminating) (copper-containing) **Comments:** A group of enzymes including those oxidizing primary monoamines, diamines and histamine. They are copper quinoproteins. One form of EC 1.3.1.15 orotate reductase (NADPH) from rat kidney also catalyses this reaction.
Suggested entry **EC1.4.3.6** **Common name:** methylamine oxidase **Reaction:** $RCH_2NH_2 + H_2O + O_2 = RCHO + NH_3 + H_2O_2$ **Other name(s):**amine oxidase; amine oxidase (pyridoxal containing); benzylamine oxidase; copper amine oxidase; Cu-amine oxidase; semicarbazide-sensitive amine oxidase; SSAO; *polyamine oxidase*; amine oxidase (pyridoxal containing) (incorrect); *diamine oxidase; diamino oxhydrase; monoamine oxidase; histamine deaminase; histamine oxidase* **Systematic name:** amine:oxygen oxidoreductase (deaminating and copper-containing) **Comments:**A group of enzymes oxidizing primary monoamines with little or no activity towards diamines, such as histamine. They are copper quinoproteins (2,4,5-trihydroxyphenylalanine quinone). Differ from EC 1.4.3.4, monoamine oxidase (deaminating), in being inactive towards secondary and tertiary tertiary amines.
New entry **EC1.4.3.x** **Common name:** diamine oxidase (deaminating) **Reaction:**histamine + $H_2O + O_2$ = imidazole-4-acetaldehyde + $NH_3 + H_2O_2$ **Other name(s):** diamine oxidase; diamino oxhydrase; histaminase; *histamine deaminase;* **Systematic name:** histamine:oxygen oxidoreductase (deaminating) (copper-containing) **Comments:** A group of enzymes oxidizing diamines, such as histamine, and also some primary monoamines but with little or no activity towards secondary and tertiary amines. They are copper quinoproteins (2,4,5-trihydroxyphenylalanine quinone).

Notes: 1. The IUBMB also contains appropriate references for each enzyme entry. These have been omitted for clarity.
2. In order to facilitate searching all names that have been used for an enzyme entry are retained in revised entries. Italics are used here to indicate those names that are ambiguous.

substrate oxidation. In some tissues it acts as a cellular adhesion protein [see 146], a process that appears to be mediated by the hydrogen peroxide produced as a result of substrate oxidation [147]. The hydrogen peroxide generated by the SSAO-catalysed amine oxidation has also been suggested to act as an important intracellular signal for glucose transporter mobilization in insulin-sensitive tissues [see 148], for the maturation of adipocytes and cardiomyocytes [see e.g., 149], as well as for the development of the extracellular matrix [see 150]. SSAO may also play a role in the regulation of cellular lipid trafficking and act as a regulator of neuronal K^+ channel currents [see 151]. That is a great deal of activity for one enzyme, and it is not known how it divides its time between these different roles as well as functioning to detoxify endogenous and xenobiotic amines.

Like MAO, diamine oxidase [152] and SSAO [see 153] are, imidazoline I_2 sites (amiloride-binding proteins). However, the plasma and endothelial-surface location of SSAO might make it more important in this respect. SSAO levels can also change quite dramatically in some disease states, being increased in diabetes (both Types 1 & 2), Alzheimer's disease and cardiac disease but decreased in burns and some cancers [see 95, 145]. There is also an

evidence for an endogenous plasma activator of SSAO [154]. Alterations in MAO activity in disease states are generally less dramatic than those reported for SSAO.

In contrast MAO seems rather single-minded in its dedication to amine oxidation, although this might merely be a reflection of our ignorance. There have been various suggestions that the hydrogen peroxide formed in the MAO-catalysed reaction may be used for other processes, including thyroxine formation in the thyroid and prostaglandin synthesis, but utilisation of products from one enzyme reaction for other processes are a central feature of cellular metabolism. As with SSAO, the hydrogen peroxide generated by MAO-catalysed amine oxidation has been suggested to act as an intracellular signal for glucose transporter mobilisation in insulin-sensitive tissues and for the maturation of adipocytes and cardiomyocytes. However, MAO appears to be less effective than SSAO in such processes [see 149], perhaps reflecting the different cellular localisations of the two enzymes or their relative activities. MAO-B substrates have also been shown to induce mitogenesis of a kidney cell line, which had been transfected to express that enzyme, as a result of H_2O_2 formation [155].

Knock-out experiments have indicated MAO activity to be important during development. A notable example is the compulsive-aggressive phenotype that arises from lack of MAO-A gene function in humans [156] and mice [157, 158]. This effect, which may reflect the importance of serotonin levels during development [see e.g., 159], can be mimicked by administration of clorgyline during the early postnatal period [160]. Studies MAO-A 'knock-out' mice have also shown the maintenance of 5-HT levels to be important for the normal development of the thalamocortical axons and the aggregation of neurones to form barrels [161, 162].

Such results indicate the difference between having an impaired enzyme during development, as compared to the effects of inhibiting MAO-A in the adult. Furthermore, the effects of more subtle alterations in MAO activity, either in the adult or during development, remain to be understood. Substantial inhibition of MAO (> 80%) in the adult rat has been shown to be necessary before of any potentiation of the effects of L- tryptophan [163] or changes to the stereotypic responses to 2-phenylethylamine [16] could be observed. However, as will be discussed elsewhere in this volume, differences in the activities of platelet MAO-B have been associated with personality phenotype [see 164].

Together with all other oxidases that produce hydrogen peroxide, which can act as a source of reactive-oxygen containing radicals, it has been suggested that MAO may be involved in cell death under some pathophysiological conditions [see 165]. Certainly, there is ample evidence for an involvement of oxidative stress in neurodegenerative diseases [see 166-168] and the mitochondrial location of MAO might result in the H_2O_2 generated by that enzyme being particularly deleterious to mitochondrial function [see 169-171]. It does appear that the activity of MAO may contribute to cell death following ischemia- reperfusion [see 172]. However, many model studies have been performed under somewhat extreme conditions and the quantitative importance of MAO, in relation to other enzymes that may form toxic, or pro-toxic, oxygen-containing products, remains to be evaluated.

CONCLUSION

The purpose of this account is to indicate what we do not know, as much as what we do know. The application of recent technological developments in polynucleotide technology and the availability of the crystal structure for MAO-B [173], with the promise that the structure for MAO-A should soon be available from at least one source [174], have allowed some major advances, but there remain several grey areas about the regulation and functions of the enzymes. There will be a lot of fun, and probably some startling new discoveries, in the MAO field for many years to come.

ACKNOWLEDGEMENTS

We are grateful to the Health Research Board and to the Science Foundation of Ireland for support.

REFERENCES

[1] Abell, C.W.; Kwan, S.W. *Prog. Nucleic Acid Res. Mol. Biol.*, **2000**, *65*, 29.
[2] Shih, J.C.; Chen, K.; Ridd, M.J. *Annu. Rev. Neurosci.*, **1999**, *22*, 197.
[3] Wouters, J. *Curr. Med. Chem.*, **1998**, 5, 137.
[4] Tipton, K.F.; O'Sullivan, J.; Youdim, M.B.H. (2004) Monoamine oxidases (MAO): Functions in the nervous system. In *Encyclopedia of Neuroscience,* Adelman, G; B. Smith, B, Eds.: Elsevier Science BV, Amsterdam, 3rd Edition, **2004**, <http://www1.elsevier.com/homepage/sah/ens/>, in press.
[5] O'Brien, E.M.; Tipton, K.F. In *Monoamine Oxidase Inhibitors in Neurological Diseases,* Lieberman, A.; Olanow, C.W.; Youdim, M.B.H.; Tipton, K.F., Eds.; Marcel Dekker, New York, **1994**, pp. 31-76.
[6] Tipton, K.F.; Singer, T.P. *J. Neurochem.*, **1993**, *61*, 1191.
[7] Houslay, M.D.; Tipton, K.F. *Biochem. J.*, **1975**, *145*, 311.
[8] Tipton, K.F.; O'Carroll, A.M.; McCrodden, J.M. J. Neural. Transm [Suppl.], **1997**, *23*, 25.
[9] Husain, M.; Edmondson, D.E.; Singer, T.P. *Biochemistry*, **1982**, *21*, 595.
[10] Pearce, L.B.; Roth, J.A. *Biochemistry*, **1985**, *24*, 1821.
[11] McDonald, A.G.; Tipton, K.F. In *Encyclopedia of Catalysis*, Horváth, I.T. Ed.; John Wiley & Sons, Inc., New York, **2003**, Vol. *4* , pp. 395-471.
[12] Ramsay, R.R.; Gravestock, M.B. *Mini Rev. Med. Chem.*, **2003**, *3*, 129.
[13] Tatton, W.G.; Chalmers-Redman, R.M. *Neurology*, **1996**, *47*, S171.
[14] Kragten, E.; Lalande, I.; Zimmermann, K.; Roggo, S.; Schindler, P.; Müller, D.; van Oostrum, J.; Waldmeier, P.; Fürst, P. *J. Biol. Chem.*, **1998**, *273*, 5821.
[15] Maruyama, W.; Akao, Y.; Carrillo, M.C.; Kitani, K.; Youdium, M.B.H.; Naoi, M. *Neurotoxicol. Teratol.*, **2002**, *24*, 675.
[16] Youdim, M.B.H.; Tipton, K.F. *Parkinsonism Relat. Disord.*, **2002**, *8*, 247.
[17] Brabeck, C.; Pfeiffer, R.; Leake, A.; Beneke, S.; Meyer, R.; Burkle, A. J. Pharmacol. Exp. Ther., **2003**, 306, 973.
[18] Seymour, C.B.; Mothersill, C.; Mooney, R.; Moriarty, M.; Tipton, K.F. *Br. J. Cancer,* **2003**, *89*, 1979.
[19] Birks, J.; Flicker, L. *Cochrane Database Syst Rev.* **2003**;(1):CD000442.
[20] Tipton, K.F. *Voprosy Med. Khim.*, **1997**, *46*, 494.
[21] Hasan, F.; Mc Crodden, J.M.; Kennedy, N.P.; Tipton, K.F. *J Neural Trans [Suppl.]*, **1998**, *26*, 1.
[22] Youdim, M.B.H.; Weinstock, M. *Neurotoxicology*, **2004**, *25*, 243.

[23] Tipton, K.F. In *Enzymology LabFax*, Engel, P.C., ED.; Bios Scientific Publishers, Oxford, UK & Academic Press, San Diego, USA, **1996**; pp. 115-174.

[24] Nelson, D.L.; Herbet, A.; Glowinski, J.; Hamon, M. J. Neurochem., **1979**, *32*, 1829.

[25] Saura, J.; Bleuel, Z.; Ulrich, J.; Mendelowitsch, A.; Chen, K.; Shih, J.C.; Malherbe, P.; Da Prada, M.; Richards, J.G. *Neuroscience*, **1996**, *70*, 755.

[26] Saura, J.; Nadal, E.; van den Berg, B.; Vila, M.; Bombi, J.A.; Mahy, N. *Life Sci.*, **1996**, *59*, 1341.

[27] Tipton, K.F. In *Nature Encyclopedia of Life Sciences*; Nature Publishing, Group London, **2001**, http://www.els.net/ [doi:10.1038/npg.els.0000601]

[28] Fowler, C.J.; Mantle, T.J.; Tipton, K.F. *Biochem. Pharmacol.*, **1982**, *31*, 3555.

[29] Avila, M.; Balsa, M.D.; Fernandez-Alvarez, E.; Tipton, K.F.; Unzeta, M. *Biochem. Pharmacol.*, **1993**, *45*, 2231.

[30] Waley, S.G. (1985) *Biochem. J.*, **1985**, *277*, 843.

[31] Gomez, N.; Unzeta, M.; Tipton, K.F.; Anderson, M.C.; O'Carroll, A.M. *Biochem. Pharmacol.*, **1986**, *35*, 4467.

[32] O'Carroll, A.M.; Anderson, M.C.; Tobbia, I.; Phillips, J.P.; Tipton, K.F. *Biochem. Pharmacol.*, **1989**, *38*, 901.

[33] Yu, P.H.; Tipton, K.F. *Biochem. Pharmacol.*, **1989**, *38*, 4245.

[34] Tipton, K.F.; Fowler, C.J.; McCrodden, J.M.; Strolin Benedetti, M. *Biochem. J.* **1983**, *209*, 235.

[35] Tipton, K.F.; McCrodden, J.M.; Youdim, M.B.H. *Biochem. J.*, **1986**, *125*, 521.

[36] O'Brien, E.M.; Tipton, K.F.; McCrodden, J.M.; Youdim, M.B.H. *Biochem. Pharmacol.*, **1994**, *47*, 617.

[37] Frederiks, W.M.; Marx, F. Histochem. J., **1985**, *17*, 707.

[38] Nakos, G.; Gossrau, R. *Acta Histochem.*, **1995**. *97*, 121.

[39] Willoughby, J.; Glover, V.; Sandler, M. *J. Neural. Transm.*, **1988**, *74*, 29.

[40] Sivasubramaniam, S.D.; Finch, C.C.; Rodriguez, M.J.; Mahy, N.; Billett, E.E. *Cell Tissue Res.*, **2003**, 313, 291.

[41] Rodriguez, M.J.; Saura, J.; Billett, E.E.; Finch, C.C.; Mahy, N. *Cell Tissue Res.*, **2001**, *304*, 215.

[42] Fowler, C.J.; Oreland, L.; Marcusson, J.; Winblad, B. *Naunyn Schmiedebergs Arch. Pharmacol.*, **1980**, *311*, 263.

[43] Riley, L.A.; Denney, R.M. *Biochem. Pharmacol.*, **1999**, *42*, 1953.

[44] Mukherjee, J., Yang, Z.Y. *Nucl. Med. Biol.*, **1999**, *26*, 619.

[45] Fowler, J.S.; Logan, J.; Volkow, N.D.; Wang, G.J.; MacGregor, R.R.; Ding, Y.S. *Methods*, **2002**, *27*, 263.

[46] Balciuniene, J.; Emilsson, L.; Oreland, L.; Pettersson, U.; Jazin, E. *Hum. Genet.*, **2002**, *110*, 1.

[47] Costa-Mallen, P.; Afsharinejad, Z.; Kelada, S.N.; Costa, L.G.; Franklin, G.M.; Swanson, P.D.; Longstreth, W.T. Jr.; Viernes, H.M.; Farin, F.M.; Smith-Weller, T.; Checkoway, H. *Mov. Disord.*, **2004**, *19*, 76.

[48] Fan. J.; Fossella, J.; Sommer, T.; Wu, Y.; Posner, M.I. *Proc. Natl. Acad. Sci. USA.*, **2003**, *100*, 7406.

[49] Norton, N.; Kirov, G.; Zammit, S.; Jones, G.; Jones, S.; Owen, R.; Krawczak, M.; Williams, N.M.; O'Donovan, M.C.; Owen, M.J. Am. J. Med. Genet. **2002**,*114*, 491.

[50] Tadic, A.; Rujescu, D.; Szegedi, A,.; Giegling, I.; Singer, P.; Moller, H.J.; Dahmen, N. *Am. J. Med. Genet.*, **2003**, *117B*, 1.

[51] Strolin Benedetti, M.; Marrari, P.; Colombo, M.; Castelli, M.G.; Arand, M.; Oesch, F.; Dostert, P. *J. Pharm. Pharmacol.*, **1994**, *46*, 814.

[52] Szutowicz, A.; Tomaszewicz, M.; Orsulak , P.J. *J. Biol. Chem.*, **1989**, *264*, 17660.

[53] Becker, R.E.; Giambalvo, C.; Fox, R.A.; Macho, M. *Science*, **1983**, *221*, 476.

[54] Egashira, T.; Takano, R.; Yamanaka, Y. *Jpn. J. Pharmacol.*, **1986**, *42*, 583.

[55] Egashira, T.; Obata, T.; Nagai, T.; Kimba, Y.; Takano, R.; Yamanaka, Y. *Biochem. Pharmacol.*, **1989**, *38*, 597.

[56] Starlinger, H. *Hoppe Seylers Z. Physiol. Chem.*, **1977**, *358*, 491.

[57] Fernandez-Novoa, L.; Pastuszko, A.; Wilson, D.F. *Biochem. Pharmacol.*, **1991**, *42*, 2351.

[58] Murphy, S.; Pastuszko, A. *Neurochem. Res.*, **1994**, *19*, 177.

[59] Naoi, M.; Maruyama, W.; Sasuga, S.; Deng, Y.; Dostert , P.; Ohta, S.; Takahashi, T. *Neurochem. Int.*, **1994**, *25*, 475.

[60] Rommelspacher, H.; May, T.; Salewski, B. *Eur. J. Pharmacol.*, **1994**, *252*, 51.

[61] Buckman, T.D; Eiduson, S.; Boscia, R. *Biochem Pharmacol.*, **1983** *32*, 3639.

[62] Naoi, M.; Ishiki, R.; Nomura, Y.; Hasegawa, S.; Nagatsu, T. *Neurosci. Lett.*, **1987**, *74*, 232.

[63] Orologas, A.G.; Buckman, T.D.; Eiduson, S. *Neurosci. Lett.*, **1986**, *68*, 293.

[64] Buckman, T.D; Kling, A.; Sutphin, M.S.; Steinberg, A.; Eiduson, S. Psychiatry Res., **1990**, *31*, 1.

[65] Meszaros, Z.; Borcsiczky, D.; Mate, M.; Tarcali, J.; Tekes, K.; Magyar, K. *J. Neural Transm. [Suppl.]*, **1998**, *52*, 79.

[66] Kaneda, Y.; Fujii, A.; Nagamine, I. *Prog. Neuropsychopharmacol. Biol. Psychiat.*, **2001**, *25*, 983.

[67] Marcolin, M.A.; Davis, J.M. *Schizophr. Res.*, **1992**, *7*, 249.

[68] Spivak, B.; Kosower, N.; Zipser, Y.; Shreiber-Schul, N.; Apter, A.; Tyano, S.; Weizman, A. *Clin. Neuropharmacol.*, **1994**, *17*, 83.

[69] Ramchand, C.N.; Gliddon, A.E.; Clark, A.E.; Hemmings, G.P. *Life Sci.*, **1995**, *56*, 1639.

[70] Fang, J.; Yu, P.H.; Gorrod, J.W.; Boulton, A.A. *Psychopharmacol. (Berl)*, **1995**, 118, 206.

[71] Fowler, J.S.; Logan, J.; Wang, G.J.; Volkow, N.D. *Neurotoxicology*, **2003** *24*, 75.

[72] Berrettini, W.H.; Vogel, W.H. (1978) *Am. J. Psychiatry.*, **1978**, *135*, 605.

[73] Vogel, W.H.; Ladman, R.K.; Berrettini, W.H. *Schizophr. Bull.*, **1980**, *6*, 232.

[74] Rose, R.M.; Castellani, S.; Boeringa, J.A.; Malek-Ahmadi, P.; Lankford, D.A.; Bessman, J.D.; Fritz ,R.R.; Denney, C.B.; Denney, R.M.; Abell , C.W. *Psychiatr. Res.*, **1986**, *17*, 141.

[75] Wise, C.D.; Potkin, S.; Bridge, P.; Wyatt, R.J. *Am. J. Psychiatry*, **1979**, *136*, 1336.

[76] Medvedev, A.E.; Glover, V. Tribulin and endogenous MAO-inhibitory regulation *in vivo*. *Neurotoxicol.*, **2004**, 25, 185.

[77] Glover, V. J. Neural Transm. [Suppl.], **1998**, 52, 307.

[78] Medvedev, A.E.; Halket, J.; Goodwin, B.L.; Sandler, M.; Glover, V. *J. Neural. Transm. Park. Dis. Dement. Sect.*, **1995**, *9*, 225.

[79] Medvedev, A.E.; Ivanov, A.S.; Kamyshanskaya, N.S.; Kirkel, A.Z.; Moskvitina, T.A.; Gorkin, V.Z.;, Li, N.Y.; Marshakov, V.Yu. Biochem. *Mol. Biol. Int.*, **1995**, *36*, 113.

[80] Clow, A.; Patel, S; Najafi, M,; Evans, P.D.; Hucklebridge, F. 1997. *Life Sci.*, **1997**, *61*, 567.

[81] Oxenkrug, G.F; McIntyre, I.M. *Life Sci.*, **1985**, *37*, 1743.

[82] McIntyre, I.M.; Norman, T.; Burrows, G.; Oxenkrug, G. *Stress Med.*, **1989**, *5*, 5.

[83] Clow, A.; Glover, V.; Oxenkrug, G.; Sandler, M. *Neurosci. Lett.*, **1989**, *107*, 331.

[84] Eglen, R.M.; Hudson, A.L.; Kendall, D.A.; Nutt, D.J.; Morgan, N.G.; Wilson, V.G.; Dillon, M.P. *Trends Pharmacol. Sci.*, **1998**, *19*, 381.

[85] Limon-Boulez, I.; Tesson, F.; Gargalidis-Moudanos, C.; Parini, A. *J. Pharmacol. Exp. Ther.*, **1996**, *276*, 359.

[86] Raddatz, R.; Parini, A.; Lanier ,S.M. *J. Biol. Chem.*, **1995**, *270*, 27961.

[87] Raddatz, R.; Parini, A.; Lanier ,S.M. *Mol. Pharmacol.*, **1997**, *52*, 549.

[88] Remaury, A.; Raddatz, R.; Ordener , C.; Savic, S,; Shih, J.C.; Chen, K.; Seif, I.; De Maeyer, E.; Lanier, S.M.; Parini, A. *Mol. Pharmacol.*, **2000**, *58*, 1085.

[89] Alemany. R.; Olmos, G.; Garcia-Sevilla, J.A. *Naunyn Schmiedebergs Arch Pharmacol.*, **1997**, *356*, 39.

[90] Tesson, F,; Limon-Boulez, I.; Urban, P.; Puype, M.; Vandekerckhove, J.; Coupry, I,; Pompon, D.; Parini, A. *J. Biol. Chem.*, **1995**, *270*, 9856.

[91] Carpene, C.; Collon, P.; Remaury, A.; Cordi, A.; Hudson, A.; Nutt, D.; Lafontan, M. *J. Pharmacol. Exp. Ther.*, **1995**, *272*, 681.

[92] Lyles, G.A. *Int. J. Biochem. Cell Biol.*, **1996**, *28*, 259.

[93] Houen, G. *APMIS Suppl.*, **1999**, *96*: 1.

[94] McGuirl, M.A.; Dooley, D.M. *Curr. Opin. Chem. Biol.*, **1999**, 3, 138.

[95] O'Sullivan, J.; Unzeta, M.; Healy, J.; O'Sullivan, M.I.; Davey, G.; Tipton, K.F. *Neurotoxicol.*, **2004**, *25*, 303.

[96] O'Sullivan, M.; Tipton, K.F.; McDevitt, W.E. Arch. Oral Biol., **2002**, 47, 399.

[97] Imamura, Y.; Kubota, R.; Wang, Y.; Asakawa, S.; Kudoh, J.; Mashima, Y.; Oguchi, Y.; Shimizu, N. *Genomics*, **1997**, *40*, 277.

[98] Zhang, X.; McIntire, W.S. *Gene*, **1996**, *179*, 279.

[99] Tipton, K.F.; Strolin Benedetti, M. In:, *Enzyme Systems that Metabolise Drugs and Other Xenobiotics,* Ioannides C, Ed.; John Wiley & Sons, Chichester, **2001**; pp. 95 –146.

[100] Hellman, N.E.; Gitlin, J.D. (2002) *Annu.R ev. Nutr.* **2002**, *22*, 439.

[101] Calabrese, L.; Carbonaro, M.; Musci, G. *J. Biol. Chem.*, **1989**, *264*, 6183.
[102] Richards, D.A. *J. Chromatogr.*, **1983**, *256*, 71.
[103] Floris, G.; Medda, R.; Padiglia, A.; Musci, G. *Biochem. Pharmacol.*, **2000**, *60*,1735.
[104] Zaitsev, V.N.; Zaitseva, I.; Papiz, M.; Lindley, P.F. *J. Biol. Inorg. Chem.*, **1999**, *4*, 579.
[105] Yang, F.M.; Friedrichs, W.E.; Cupples, R.L.; Bonifacio, M.J.; Sanford, J.A.; Horton, W.A.; Bowman, B.H. *J. Biol. Chem.*, **1990**, *265*, 10780.
[106] Patel, B.N.; David, S. *J. Biol. Chem.*, **1997**, *272*, 20185-20190.
[107] Jeong, S.Y.; David, S. *J. Biol. Chem.*, **2003**, *278*, 27144.
[108] Loeffler, D.A.; LeWitt, P.A.;. Juneau, P.L.; Sima, A.A.F.; H.-U.; Nguyen, H.-U,; DeMaggio, A.J.; Brickman, C.M.; Brewer, G.J.; Dick, R.D.; Troyer, M.D.; Kanaley, L. *Brain Res.*, **1996**, *738*, 265.
[109] Gutteridge, J.M.C.; Stocks, J. *CRC Crit. Rep. Clin. Lab. Sci.*,, **1981**, *14*, 257.
[110] Harris, Z.L. *J. Neurol. Sci.*, **2003**, *207*, 108.
[111] Johnson, S. *Med. Hypotheses*, **2001**, *56*, 171.
[112] Hammons, G.J.; Milton, D.; Stepps, K.; Guengerich, F.P.; Tukey, R.H.; Kadlubar, F.F. *Carcinogenesis.*, **1997**, *18*, 851.
[113] Pfu, W.; Marquard, H. *Toxicology*, **2001**, *166*, 25.
[114] Thompson, C.M.; Capdevila, J.H.; Strobel, H.W. J. Pharmacol. Exp. Ther., **2000**, *294*, 1120.
[115] Dupont, H.; Davies, D.S.; Strolin Benedetti, M. *Biochem. Pharmacol.*, **1987**, *36*, 1651.
[116] Yu, A.M.; Granvil, C.P.; Haining, R.L.; Krausz, K.W.; Corchero, J.; Kupfer, A.; Idle, J.R.; Gonzalez, F.J. *J. Pharmacol. Exp. Ther.*, **2003**, *304*, 539.
[117] Constantino, L.; Paixao, P.; Moreir, R.; Portela, M.J.; Do Rosario, V.E.; Iley, J. *Exper. Toxicol. Pathol.*, 1999, *51*, 299.
[118] Rochat, B.; Kosel, M.; Boss, G.; Testa, B.; Gollet, M.; Baumann, P. *Biochem. Pharmacol.*, **1998**, *56*, 15.
[119] Fowler, J.S.; Ding, Y.S.; Logan, J.; MacGregor, R.R.; Shea, C.; Garza, V.; Gimi, R.; Volkow, N.D.; Wang, G.J.; Schlyer, D.; Ferrieri, R.; Gatley, S.J.; Alexoff, D.; Carter, P.; King, P.; Pappas, N.; Arnett, C.D. *Nucl. Med. Biol.*, **2001**, *28*, 779.
[120] Sullivan, J.P.; Tipton, K.F. *Neurochem. Res.*, **1992**, *8*, 791.
[121] Inoue, H.; Castagnoli, K.; Van Der Schyf, C.; Mabic, S.; Igarashi, K.; Castagnoli, N. *J. Pharmacol. Exp. Ther.*, **1999**, *291*, 856.
[122] O'Brien, E.M.; Dostert, P.; Tipton, K.F. *Biochem. Pharmacol.*, **1995**, *50*, 317.
[123] Reid, A.A.; Hill, J.L.; Murphy, D.L. *Naunyn-Schmiedebergs Arch. Pharmacol.*. 1988, *388*, 678.
[124] Egashira, T.; Takayama, F., Yamanaka, Y. *Jpn. J. Pharmacol.*, **1999**, *81*, 115.
[125] Krueger, M.J.; Mazouz, F.; Ramsay, R.R.; Milcent, R.; Singer, T.P. *Biochem. Biophys. Res. Commun.*, **1995**, *206*, 556.
[126] Garrick, N.A.; Murphy, D.L. *Psychopharmacol. (Berl).*, **1980**, *72*, 27.
[127] Garrick, N.A.; Murphy, D.L. *Biochem. Pharmacol.*, **1982**, *31*, 4061.
[128] O'Carroll, A.M.; Bardsley, M.E.; Tipton, K.F. *Neurochem. Int.*, **1986**, *8*, 493.
[129] Figueiredo, I.V.; Caramona, M.; Paiva, M.Q.; Guimaraes, S. *J. Auton. Pharmacol.*, **1998**, *18*, 123-128.
[130] Branco, D.; Caramona, M.; Martel, F.; de Almeida, J.A.; Osswald, W. Naunyn *Schmiedebergs Arch. Pharmacol.*, **1992**, *346*, 286.
[131] O'Carroll, A.M.; Fowler, C.J.; Phillips, J.P.; Tobbia, I.; Tipton, K.F. *Naunyn Schmiedebergs Arch. Pharmacol.*, **1983**, *322*, 198.
[132] Houslay, M.D.; Tipton, K.F. *Biochem. J.*, **1974**, *139*, 645.
[133] Strolin Benedetti, M.; Sanson, G.; Bona, L.; Gallina, M.; Persiani, S.; Tipton, K.F. *J. Neural Transm. [Suppl.]*, **1998**, *52*, 233.
[134] Yu, P.H.; Bailey, B.A.; Durden, D.A.; Boulton, A.A. *Biochem. Pharmacol.*, **1986**, *35*,1027.
[135] Gesi, M.; Santinami, A.; Ruffoli, R.; Conti, G.; Fornai. F. *Pharmacol. Toxicol.*, **2001**, *89*, 217.
[136] Lizcano, J.M.; Tipton, K.F.; Unzeta, M. *Biochem. J.*, **1998**, *331*, 69.
[137] Gewitz, M.H.; Gillis, C.N. *J. Appl. Physiol.*, **1981**, *50*, 118.
[138] Elliott , J.; Callingham, B.A.; Sharman, D.F. *Br. J. Pharmacol.*, **1989**, 98, 507.
[139] Callingham, B.A.; McCarry, W.J.; Barrand, M.A. *In Monoamine Oxidase and Disease. Prospects for Therapy with Reversible Inhibitors,* Tipton, K.F,; Dostert, P.; Strolin Benedetti, M., Eds.; Academic Press: London, **1984**, pp. 595–596.
[140] Fitzgerald,, D.H.; Tipton, K.F. *J. Neural Transm.*, **2002**, *109*, 251.
[141] Dumpty, H. cited by Carroll, L. In *Through the Looking-glass,* **1871** . Chapter 6, 1st or any subsequent edition.
[142] Enzyme Nomenclature < http://www.chem.qmul.ac.uk/iubmb/enzyme/>, **2004**.
[143] Enzyme Rules <http://www.chem.qmul.ac.uk/iubmb/enzyme/rules.html>, **2004**.
[144] Enzyme Nomenclature (1964) In Enzyme Nomenclature . Recommendations (1964) of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes, Together with their Units and the Symbols of Enzyme Kinetics, Elsevier: Amsterdam, **1965**.
[145] Tipton, K.F.; O'Sullivan, M.I.; Davey, G.P.; O'Sullivan, J. *Biochem. Soc. Trans.*, **2003**, *31*, 711.
[146] Jalkanen, S.; Salmi, M. (2001) *EMBO J.*, **2001**, *20*, 3893.
[147] O'Sullivan, J.; O'Sullivan, M.; Tipton, K.F.; Unzeta, M.; Hernandez, M.; Davey, G *Biochim. Biophys. Acta*, **2003**,*1647*, 367.
[148] Zorzano, A.; Abella, A.; Marti, L.; Carpene, C.; Palacin, M.; Testar, X. *Biochim. Biophys. Acta*, **2003**, *1647*, 3.
[149] Mercier, N.; Moldes, M.; El Hadri, K.; Feve, B. *Biochem. J.*, **2001**, *358*, 335.
[150] Langford, S.D.; Trent, M.B.; Boor, P.J. *Cardiovasc. Toxicol.*, **2002**, *2*, 141.
[151] Nocera, S.; Marcocci, L.; Pietrangeli, P.; Mondovi, B. *Amino Acids*, **2003**, *24*, 13.
[152] Novotny, W.F.; Chassande, O.; Baker, M.; Lazdunski, M.; Barbry, P. *J. Biol. Chem.*, **1994**, *269*, 9921.
[153] Mu, D.; Medzihradsezky, K.F.; Adams, G.W.; Mayer, P.; Hiness, W.M.; Burlingames, A.L.; Smith, A.J.; Cai, D.; Klinman, J.P. *J. Biol. Chem.*, **1994**, *269*, 9926.
[154] Dalfo, E.; Hernandez, M.; Lizcano, J.M.; Tipton, K.F.; Unzeta, M. *Biochim. Biophys. Acta*, **2003**, *1638*, 278.
[155] Vindis, C.; Seguelas, M.H.; Bianchi, P.; Parini, A.; Cambon, C. *Biochem. Biophys. Res. Commun.*, **2000**, *271*, 181.
[156] Brunner, H.G.; Nelen, M.; Breakefield, X.O.; Ropers, H.H.; van Oost, B.A. (1993). *Science*, **1993**, *262*, 578.
[157] Cases, O.; Seif, I.; Grimsby, J.; Gaspar, P.; Chen, K.; Pournin, S.; Muller, U.; Aguet, M.; Babinet, C.; Shih, J.C.; De Maeyer, E. *Science*, **1995**, *268*, 1763.
[158] Holschneider, D.P.; Chen, K.; Seif, I.; Shih, J,C. *Brain Res Bull.*, **2001**, *56*, 453.
[159] Gingrich, J.A.; Ansorge, M.S.; Merker, R.; Weisstaub, N.; Zhou, M. *CNS Spectr.*, **2003**, *8*, 572.
[160] Boylan, C.B.; Kesterson, K.L.; Bennett-Clarke, C.A.; Chiaia, N.L.; Rhoades, R.W. *Brain Res. Dev. Brain Res.*, **2001**, *129*, 95.
[161] Cases, O.; Vitalis, T.; Seif, I.; De Maeyer, E.; Sotelo, C.; Gaspar. P. *Neuron*, **1996**,*16*, 297.
[162] Rebsam, A.; Seif, I.; Gaspar, P. *J. Neurosci.*, **2002**, *22*, 8541.
[163] Green, A.R.; Youdim, M.B.H. (1975) *Br. J. Pharmacol.*, **1975**, *55*, 415.
[164] Oreland, L. *Neurotoxicology*, **2004**, *25*, 79.
[165] De Zutter, G.S.; Davis, R.J. Proc. Natl. Acad .Sci. USA, **2001**, *98*, 6168.
[166] Behl, C.; Moosmann, B. *Biol. Chem.*, **2002**, *383*, 521.
[167] Foley, P.; Riederer, P. J. Neurol., **2000**, *247 Suppl 2*, II82.
[168] Jenner, P.; Olanow, C.W. *Neurology*, **1996**, *47 [Suppl 3]*, S161-70.
[169] Sandri, G.; Panfili, E.; Ernster, L.. *Biochim, Biophys, Acta*, **1990**, *1035*, 300.
[170] Cohen, G.; Kesler, N. *J. Neurochem.*, **1999**, 73, 2310.
[171] Mattson, M.P.; Liu. D. Neuromolecular Med., **2002**, *2*, :215.
[172] Kunduzova, O.R.; Bianchi, P.; Parini, A.; Cambon, C. *Eur. J. Pharmacol.*, **2002**, *448*, 225.
[173] Binda, C.; Newton-Vinson, P.; Hubalek, F.; Edmondson, D.E.; Mattevi, A. *Nat. Struct. Biol.*, **2002**, *9*, 22.
[174] Ma, J.; Kubota, F.; Yoshimura, M.; Yamashita, E.; Nakagawa, A.; Ito, A.; Tsukihara, T. *Acta Cryst.*, **2004**, *D60*, 317.

Monoamine oxidase (MAO): functions in the central nervous system

Keith F. Tipton, Jeff O'Sullivan and Moussa B.H. Youdim

1. Biochemistry

Monoamine oxidase (monoamine oxygen oxidoreductase [deaminating] [flavin-containing], EC 1.4.3.4; MAO) oxidizes primary aliphatic and aromatic amines and some secondary and tertiary amines. The reaction can be represented by the following generalized equation:

$$RCH_2NR'R'' + O_2 + H_2O \rightarrow RCHO + NR'R'' + H_2O_2$$

Two isoenzymes of monoamine oxidase are present in most mammalian tissues. These enzymes, MAO-A and -B, were originally distinguished by their sensitivities to inhibition by the acetylenic inhibitors clorgyline and deprenyl and by their substrate specificities. Typically MAO-A is inhibited by low concentrations of clorgyline and catalyzes the oxidative deamination of 5-hydroxytryptamine (5-HT), whereas MAO-B is inhibited by low concentrations of (-)-deprenyl (selegiline) and is active toward benzylamine and 2-phenylethylamine (PEA). These substrate specificities are not absolute. For example, 5-HT is a substrate for MAO-B as well as MAO-A in rat brain and liver. However, in the rat brain the K_m and V_{max} values for MAO-B are six-fold higher and nine-fold lower, respectively, than those for MAO-A. Hence, relatively low concentrations of 5-HT will be oxidized essentially by MAO-A alone, whereas at very high concentrations the B form can contribute about 10% of the total activity. Similarly, PEA is a substrate for MAO-A but with a considerably higher K_m and a V_{max} value that is five- to six-fold lower than the corresponding values for MAO-B. Thus, although the A form can contribute to a percentage of the total activity at very high concentrations of PEA, only the activity of the B form is important at low concentrations of this substrate. In rat heart, which contains very little MAO-B, it has been reportedly possible to detect low levels of benzylamine oxidation by MAO-A. These factors are important in interpreting the results of attempts to determine the relative proportions of the two enzymes in tissues from their activities toward such substrates.

In most species examined, tyramine is a substrate for both enzymes, as are dopamine and noradrenaline in the human brain. Although tyramine has been frequently regarded as a substrate for both forms of MAO, the kinetic parameters toward this substrate differ. With the enzymes from rat liver mitochondria, for example, the K_m and V_{max} values of MAO-A toward this substrate were found to be 107 μM and 11 nmol.min^{-1}. mg protein^{-1}, respectively, whereas the values for MAO-B were 579 μM and 20 nmol.min^{-1}.mg protein^{-1}, respectively. Therefore, the proportions of the total activity toward tyramine contributed by each of the two forms will depend to some extent on the concentration of substrate. Thus about 32% of the total rat liver MAO activity toward 50 μM tyramine will be due to MAO-B, but this proportion rises to 57% at a tyramine concentration of 1 mM. Hence, there is no simple subdivision into specific MAO-A, specific MAO-B, and mixed substrates but rather a continuum.

The selectivities of the monoamine oxidases for a range of substrates are summarized in Table 1. These refer to the enzymes from human and rat brain. However, species differences may exist between the specificities of the enzymes, so it cannot be assumed that the behavior will necessarily be identical in all tissues and species. Indeed marked species differences have been found for the selectivities of MAO-A and -B for a series of derivatives of milacemide (O'Brien et al., 1995) and rigid analogues of MPTP (Krueger et al., 1995).

The native enzyme appears to have a molecular weight of about 100 kDa and to be composed of two identical subunits. The cDNAs for human, ox, and rat MAO-A encode polypeptides of 527 amino acid residues corresponding to molecular weights of 59.7 kDa, 59.8 kDa, and 58.3 kDa, respectively. Similarly the cDNAs for human and ox MAO-B encode proteins of 520 amino acid residues with molecular weights of 58.8 kDa and 58.4 kDa, respectively. Transfection of cDNAs for MAO-A or -B into COS cells (a transformed fibribroblast-like cell line from the African green monkey, Cercopithecus aethiops, kidney), which have very low endogenous MAO activity, has shown them to confer the corresponding activity in these cells.

Comparison of the sequences of the different forms with those from other species shows that the degree of similarity between the same form from different species is greater than between different forms from the same species. For example, ox and human MAO-B have approximately 90% of amino acid residues identical, whereas the overall identity of amino acid sequences for MAO-A and -B from the same species is about 70%. Earlier reports that MAO was a glycoprotein have not been confirmed. Examination of the primary structures of MAO-A and -B also suggests that these proteins are not extensively glycosylated. Only one potential N-glycosylation site exists in each protein, with each site in a different relative position. Similarly, reports that the enzyme contained copper or iron that was essential for activity have been shown to be incorrect.

Flavin-adenine dinucleotide (FAD is covalently bound by a thio-ether bridge from the 8a carbon to a cysteine residue in the enzyme. The pentapeptide sequence Ser-Gly-Gly-Cys-Tyr, which includes the FAD-binding cysteine residue, has been shown to be conserved in MAO preparations from several species and to be located in the C-terminal region of both MAO-A and -B proteins. The covalent attachment of the FAD appears to be an autocatalytic process. Substrate oxidation is believed to involve the formation of an adduct of the substrate with position 4a of the isoalloxazine ring of the FAD. Attack by the adjacent N(5) on this 4a-alkylated intermediate then results in the release of

the corresponding imine, which is hydrolyzed to the aldehyde, and the reduced flavin cofactor, which is reoxidized by oxygen (Miller and Edmondson, 1999). In this model the functions of the amino acid residues at the active site are essentially to locate the substrate in the correct orientation and to provide an appropriate environment for the flavin catalyzed reaction to occur.

The crystal structure of MAO-B has been solved (Binda et al., 2002), but that of MAO-A is not yet available. However, comparative studies have suggested that the active sites and overall tertiary structures of MAO-A and -B may be quite similar. The structural studies give an explanation for the tight association of the enzyme with the mitochondrial outer membrane by showing it to be bound through a C-terminal transmembrane helix together with apolar loops that are located at various positions in the sequence. The active site of MAO-B consists of a 420 Å3-hydrophobic substrate cavity interconnected to an entrance cavity of 290 Å3. The recognition site for the substrate amino group is an aromatic cage formed by two tyrosine residues. The difference between the specificities of MAO-A and -B may result from different geometries of the active site and particularly the residues that have been identified in MAO-B as separating the substrate cavity from the entrance cavity. Structural illustrations of MAO-B can be found at the Protein Data Bank (PDB) (http://www.rcsb.org/pdb) by entering the PDB identifier 1GOS or the name monoamine oxidase

2. Distribution

Sub-cellular fractionation studies indicate that both MAO-A and -B are tightly associated with the mitochondrial outer membrane. Indeed these enzymes have often been used as markers for this particular cellular compartment. However, significant levels of both MAO-A and -B activities have been found in the microsomal fractions. The properties of the microsomal enzyme appear to be similar to those of the mitochondrial enzyme. The observation that the enzyme associated with the microsomal fraction has a faster turnover rate than the mitochondrial enzyme led to the suggestion that it might represent newly synthesized enzyme, that is, a precursor of mitochondrial MAO not yet inserted into the mitochondrial membrane. However, this seems unlikely because MAO from rat liver has been reported to be synthesized in vitro on cytosolic free polysomes rather than in the endoplasmic reticulum.

MAO has an almost ubiquitous occurrence in the cells of most mammalian species, the most notable exceptions being erythrocytes. Several tissues from different species express essentially only one form of MAO (see Figure 1), for example MAO-A predominates in human placenta and rat spleen whereas MAO-B dominates in human blood platelets, ox liver and kidney, and pig liver.

MAO-A and -B are present in approximately equal amounts in rat and human liver. In the rat peripheral nervous system histochemical studies have shown MAO to be localized in the endothelial cells of the endoneurial vessels, the Schwann cells, and the neurons in some unmyelinated axons. Microvessels from the blood-brain barrier are rich in MAO-B activity, but a comparison between six mammalian species showed a 25-fold difference in the amounts of enzyme present

The enzymes are rather evenly distributed in the different regions of the human brain. Positron emission studies in human brain in vivo after intravenous injection of the ^{11}C-labeled inhibitors, clorgyline and (-)-deprenyl showed the anatomical distribution of ^{11}C to parallel the distribution of MAO-A and -B activities in human brain autopsy material.

Immunohistochemical studies using specific polyclonal and monoclonal antibodies have shown that serotonergic neurons (e.g., cells of the nucleus raphe dorsalis, nucleus centralis superior, and glial astrocytes) contain predominantly MAO-B and catecholaminergic neurons (e.g., cells of the substantia nigra, locus coeruleus, nucleus subcoeruleus, and the periventricular regions of the hypothalamus) contain predominantly MAO-A. Data obtained with mRNA probes are in general agreement with these results.

Studies on the effects of specific uptake inhibitors on amine metabolism in human brain synaptosomes showed that caudatal dopaminergic nerve endings contained only MAO-A. In contrast, MAO-A appears to be absent from serotonergic neurons. Recently it has been reported that there are two types of mitochondria in serotoninergic neurons, which differ in the presence of MAO. The neuronal cell bodies and dendrites contain both monoamine oxidase-B positive and monoamine oxidase negative mitochondria. In contrast, only mitochondria lacking MAO-B were present in the serotoninergic nerve terminals (Arai et al., 2002). Some caution is, however, necessary in extrapolating results obtained from one species to another because it appears, for example, that only MAO-A is involved in dopamine metabolism in the rat striatum, but both MAO-A and -B can contribute, at least at higher dopamine levels, in the mouse (Fornai et al., 1999).

3. Regulation and turnover

MAO-A and -B differ markedly in their patterns of developmental expression and genetic regulation. In most mammalian species MAO-A appears before MAO-B. Changes in MAO activities during development probably reflect events of cellular differentiation as well as the changing ratios of different cell types within tissues. In the developing rat central nervous system the increase in MAO-A follows the caudal-rostral pattern of neuronal differentiation. MAO-B activity is at relatively high levels in many tissues at birth, especially the liver. However, the level of this enzyme increases dramatically in the brain after birth, probably because of the proliferation of astrocytes.

The process of aging also appears to involve changes in levels of MAO activity. When human skin fibroblasts were cultured from males in the age range of 1 to 60 years, the specific activity of MAO-A assessed during proliferative growth of these cells showed an overall increase of 5- to 10-fold with the age of the donor. Increases in human brain MAO-B activity with age have been observed in several studies, possibly as a result of glial cell proliferation. Results on the effects of age on MAO-A in the human brain have been conflicting in that both increases and no change have been reported. In contrast there is essentially no change in the MAO-A or -B activities in the rat central nervous system from 2 to 28 months of age. In the rat heart the increase in MAO-A with age has been show to result from an age-dependent decrease in the rate of enzyme degradation without any significant change in the rate of enzyme synthesis. However, no information is available as to whether alterations in enzyme degradation rate play a role in variations of the enzyme levels in other tissues.

Both genes for MAO-A and -B have been located on the X-chromosome (Xp11.2-11.4). The two genes consist of 15 exons with identical exon-intron organization; the high degree of sequence identity suggests that the genes for MAO-A and -B are derived from a common progenitor gene. Indeed, the evidence for distinct MAO-A and -B activities in the toad indicates that duplication of the precursor MAO gene occurs fairly early in evolution. The maximal promoter activities for the MAO-A and MAO-B genes have been located in the -206/-60 and -246/-99 regions, respectively. Both of these are GC-rich regions with about 60% sequence identity. The core promoter region for MAO-A is composed of two 90-bp repeats, each of which contains two recognition elements for the Sp1 transcription factor, but with no TATA box. The MAO-B core promoter region contains two sets of overlapping Sp1 sites, which flank a CACCC element upstream of a TATA box (Shih et al., 1994). These differences in the promoter regions of the two genes may explain, at least in part, the differential expression of the MAO-A and MAO-B genes.

MAO-A but not MAO-B activity is elevated in reproductive tissue when levels of progesterone are high. In contrast, estrogen-treated ovariectomized female rats show increased MAO-B activity and decreased MAO-A activity in the cerebellum and in certain areas of the brain stem. Castration leads to a three-fold increase in MAO-A activity with no change in the MAO-B activity in man, and this increase can be prevented by administration of estradiol or testosterone. Thyroxine and adrenal corticosteroids both appear to influence MAO-A and -B levels. Studies with cultured human skin fibroblasts revealed that short (6 hour) exposures to several hormones, including progesterone, testosterone, tri-iodothronine, and the glucocorticoid dexmethasone, reduced the total MAO activity in these cells. In contrast progesterone, testosterone, corticosterone, and dexmethasone were found to increase the synthesis of MAO-A, without affecting MAO-B, in capillary endothelial cells.

The expression of MAO-B, but not MAO-A, is regulated via a mitogen-activated protein kinase (MAPK) pathway that includes protein kinase C (PKC), Ras, MEK1, MEK3, MEK7, ERK2, JNK1, and p38/RK. The responsive promoter region is an overlapping binding site for members of the Sp family of transcription factors, Sp1/Egr-1/Sp1, located between nucleotides -239 and -227. This family of proteins has similar structural features and a conserved zinc finger DNA-binding domain that can recognize the GC box (GGGGCGGGC) and/or the GT motif (GGGTGTGGC) similar affinities. Sp1 or Sp4 can transactivate the promoter activity, whereas this can be repressed by the overexpression of the transcription factors Sp3 or BTEB2 (Wong et al., 2002).

When MAO is inactivated by an irreversible inhibitor, the rate of recovery of enzyme activity can be used to estimate the rate of turnover of the enzyme in the tissues. This approach has shown MAO-A and -B in rat liver to have similar half-lives, in the range 2.5 to 3.5 days. The half-life in rat brain was found to be about 10 to 13 days, and in heart from the same species the half-life of MAO-A increases with age. The rates of turnover of the two forms of MAO in rat intestine were found to be different, with the half-life values of 2.2 and 7.5 days being reported for MAO-A and -B, respectively. In a study of the turnover of MAO in baboon brain using positron-emission tomography of the deprenyl-labeled enzyme, a half-life of about 30 days was determined, and the recovery of MAO-B in the human brain appears to be even slower. Such a slow rate of turnover in the primate brain would have important implications for the "wash-out" periods used in studies on the effects of irreversible monoamine oxidase inhibitors in the clinical situation. The half-lives of MAO are shown in Figure 2.

The activity of MAO may also play important roles during development. The possible role of MAO-A in maintaining normal 5-HT levels is discussed later in terms of aggression. Studies MAO-A "knock-out" mice have shown the maintenance of 5-HT levels is also important for the development of the whisker barrel in the mouse (Rebsam et al., 2002). Furthermore, hydrogen peroxide, which arises, in part, from amine oxidation, may be important for the maturation of adipocytes and, perhaps, some other cell types.

4. Inhibitors

Some inhibitors of MAO are shown in Figure 3. Many of the earlier MAO inhibitors reacted with the enzyme irreversibly and showed little ability to discriminate between MAO-A and -B. Hydrazine derivatives were the first irreversible inhibitors of MAO to be recognized and developed as antidepressants. Those that have been used clinically include iproniazid, phenelzine, nialamide, and isocarboxazid. However, the use of many of these was discontinued because of hepatotoxicity, which appears to have resulted from interactions with co-administered barbiturates at the metabolic level. The discovery that tranylcypromine inhibited MAO led to the development of other cyclopropylamines such as Lilly 51641 and 5-phenoxyl-3-(N-cyclopropyl)ethylamine-1,2,4-oxadiazole (PCO), which are selective for MAO-A, and Lilly 54761, which is a selective inhibitor of MAO-B. The acetylenic derivatives (-)-deprenyl, rasagiline, and to a lesser extent pargyline are selective toward MAO-B, whereas clorgyline is selective toward MAO-A. In no case is the selectivity of these inhibitors absolute and administration of sufficiently high doses results in inhibition of both MAO-A and -B. This should be kept in mind when considering some reports that suggest that some MAO-B inhibitors may have antidepressant actions. The possibility of species differences should also be considered when trying to extrapolate from animal studies to the situation in humans. An unexpected example of this was the discovery that clorgyline, which binds irreversibly to rat and human brain MAO-A following in vivo administration, does not do so in the baboon or rhesus monkey brain (Fowler et al., 2001).

Harmine and harmaline are selective, potent, and reversible inhibitors of MAO-A that belong to the harmala alkaloid class of inhibitors. The tritiated derivative of harmaline has been used as an appropriate ligand for the estimation of MAO-A active centers. Tetrahydro-ß-carbolines, while being structurally similar to the harmala alkaloids, are found in various tissues and fluids of animals and man and can be considered as potential endogenous MAO inhibitors. These compounds can be obtained by the reaction of an aldehyde or an α-keto acid with a phenolic or indole ethylamine. Most tetrahydro-ß-carbolines studied have been found to be selective inhibitors of MAO-A. In many MAO substrates the replacement of a hydrogen in the a-position to the nitrogen atom with a methyl group has resulted in the formation of MAO-A selective reversible inhibitors. Such compounds include amphetamine and α-methylbenzylamine. In addition, the selectivity of these α-methyl substituted monoamines can be considerably improved by resolution of the two optical isomers.

A number of the oxazolidinone derivatives, including cimoxatone and toloxatone, have been shown to be selective inhibitors of MAO-A. Very few useful reversible MAO-B selective inhibitors have been reported in the literature. However, simple analogues of the MAO-B substrate, benzylamine, such as benzyl alcohol, benzyl cyanide, and 4-cyanophenol have been shown to be MAO-B selective inhibitors in vitro, as have the aliphatic alcohols butan-1-ol, hexan-1-ol, and octan-1-ol. More interestingly from the pharmacological point of view, the antidepressant caroxazone has been reported to be a MAO-B selective reversible inhibitor but the selectivity toward this form of the enzyme is only five-fold. The tricyclic antidepressants imipramine and amitriptyline also show some MAO-B selectivity, and the MAO-B inhibitory properties of these compounds have been correlated with the relief of certain types of anxiety and normalization of sleep patterns in clinical trials.

The compounds brofaromine and moclobemide are reversible and selective inhibitors of MAO-A that have developed as antidepressants. They differ in their modes of action in that brofaromine is a tight-binding inhibitor, whereas it appears that it is a metabolite of moclobemide that is the effective inhibitor in vivo. A study of the possible metabolites of moclobemide led to the discovery of Ro 16-6491, which is a high-affinity reversible selective inhibitor of MAO-B.

Components of cigarette smoke have been shown to inhibit MAO irreversibly, both in the central nervous system and the periphery. Nicotine does not contribute to this effect, but it appears that several components, including cotinine (Berlin et al., 2000) (which has been measured in plasma as an indirect index of smoked tobacco); 2-naphthylamine; and 2,3,6-trimethyl-1,4-naphthoquinone; may contribute to the inhibition.

5. Physiological roles

MAO-A and/or -B in peripheral tissues such as the intestine, liver, lung, and placenta appear to play a protective role in the body by oxidizing amines from blood or preventing their entry into the circulation. Intestinal MAO may be responsible for the inactivation of vasoactive amines such as noradrenaline, tyramine, dopamine, and 5-HT of dietary origin. A number of xenobiotic amines including mescaline, milacemide, 2-phenylethanolamine , 2-phenylpropanolamine, and primaquine can be oxidatively deaminated by MAO. Liver MAO-A and -B may be involved in controlling blood levels of pressor amines that have escaped deamination by MAO in the platelets or in the gut. MAO-A in the vascular endothelial cells of the lung is believed to be important in eliminating freely circulating 5-HT from the circulation, thereby protecting the heart and vascular system from the effects of that amine. MAO-A in the syncitial trophoblast layer of the placenta and MAO-B in the microvessels of the blood-brain barrier presumably have similar protective functions because these locations constitute metabolic barriers. Consistent with this are the observations that drugs that inhibit MAO induce extreme sensitivity to the dietary amine tyramine, and pargyline infusion into amniotic fluid results in abortion.

In the central and peripheral nervous system, intraneuronal MAO-A and -B have been suggested to protect neurons from exogenous amines and/or to regulate levels of amines synthesized within a neuron. The absence or very low levels of MAO-A in serotonergic neurons

indicates that MAO-A within these neurons might not play a major role in degrading 5-HT. The low affinity of MAO-B for the endogenous transmitter suggests that, rather than limiting cytoplasmic levels of 5-HT, the major role of MAO-B in serotonergic neurons may be to eliminate foreign amines and minimize their access to synaptic vesicles. Hence, MAO-B in serotonergic neurons appears capable of enriching the neuroplasmic compartment with 5-HT, relative to many other amines and, in conjunction with membrane and vesicle 5-HT uptake systems, may contribute to the purity of the neurotransmitter delivered to the synaptic cleft. Noradrenergic neurons contain both MAO-A and -B. Furthermore, noradrenaline is a reasonably good substrate for both forms of the enzyme. However, because the affinity of the vesicular uptake mechanism for noradrenaline is much higher, uptake of noradrenaline into synaptic vesicles should be strongly favored over degradation by MAO-A or -B. An important role for MAO-A in the deamination of noradrenaline and dopamine in synaptosomes from the hypothalamus and striatum, respectively, has been reported. Extraneuronal MAO functions in the inactivation of amines that are taken up by non-neuronal cells such as glial cells. Indeed studies on the cyclic AMP-induced flux of dopamine indicate a significant extraneuronal metabolism of this amine by MAO-B.

Although the role of monoamine oxidase in terminating the actions of the amine neurotransmitters and dietary amines has been extensively studied, less attention has been paid to the functions of the products formed in the MAO reactions; that is, hydrogen peroxide, aldehyde and, in the case of primary amines, ammonia. The hydrogen peroxide formed may have signaling functions but could also act as a potential source of toxic oxygen radicals; it has been suggested that this may contribute to the destruction of the nigro-striatal dopaminergic neurons in Parkinson's disease. In the ox thyroid gland the microsomal component of MAO has been suggested to exist to produce the hydrogen peroxide needed for incorporation of iodine into thyroxine. The hydrogen peroxide resulting from MAO-catalyzed substrate oxidation has been shown to mimic the action of insulin, in cardiomyocytes and some other cell types, by increasing the number of glucose transporters at the cell surface, but the physiological significance of this is unknown. It has been suggested that the aldehydes derived from 5-HT and noradrenaline deamination may be involved in the regulation of sleep. Such aldehydes may also form adducts with amine groups, to form compounds such as tetrahydropapaveroline and peptide adducts, which may have patho-physiological roles.

6. Clinical importance

6.1. Depression

The early observation of the mood-elevating properties of MAO inhibitors led them to be termed psycho-energizers rather than antidepressants. MAO inhibitors have been reported to be particularly valuable in the treatment of anxiety in which phobia, obsessional, and panic states predominate. This action of monoamine oxidase inhibitors and the antidepressant effects of the inhibitors of presynaptic amine uptake gave rise to the amine hypothesis of affective disorders.

On the basis of this hypotheses it was predicted that MAO-A selective and non-selective MAO inhibitors that increase the concentration of 5-HT and noradrenaline in the brain should be antidepressant in nature. However, considerable (more than 80%) MAO inhibition is required before changes in brain monoamine concentration and antidepressant effects are found. Clorgyline, an irreversible MAO-A selective inhibitor, was shown to have antidepressant action. The use of clorgyline and other MAO-A selective or nonselective irreversible MAO inhibitors has been limited because individuals taking these drugs become susceptible to amines, such as tyramine, in the diet that are normally degraded by MAO in peripheral tissues, with the intestinal enzyme probably making the major contribution. When MAO is inhibited, ingested tyramine can enter the blood, from which it is taken up by adrenergic nerve terminals releasing stored noradrenaline and resulting in a hypertensive response. Lists have been compiled of foods and beverages high in tyramine, but it should be noted that the tyramine content of many foods and beverages can be extremely variable. Nonetheless, on the basis of the high concentrations of tyramine in some cheeses, this hypertensive response to dietary amines is widely known as the "cheese reaction." Longer-term administration of such inhibitors can lead to hypotension, possibly as a result of the accumulation of false transmitters in the nerves.

Most studies have shown the selective MAO-B inhibitor (-)-deprenyl to be without effect in patients suffering from endogenous depression. (-)-Deprenyl has been shown to have a very low interaction with tyramine given either orally or intravenously. Indeed the human intestinal MAO is predominantly the A-form, and thus tyramine oxidation by this tissue is not significantly affected by MAO-B inhibition. (-)-Deprenyl is not only without effect on the dietary tyramine-induced release of noradrenaline from rat cortical slices, but it antagonizes the increased noradrenaline efflux found after clorgyline treatment. It has also been suggested that (-)-deprenyl may block the uptake of tyramine into nerve endings or that it may have a direct antagonistic effect on the tyramine-induced release of noradrenaline.

The use of reversible MAO inhibitors provided an alternative solution to the problem of the "cheese reaction". If these inhibitors are competitive with respect to the amine substrate, high concentrations of a substrate such as tyramine would be expected to displace the inhibitor from the enzyme. Thus it should be possible to select a dosage of inhibitor that would give substantial inhibition of MAO but would not prevent the metabolism of high concentrations of dietary tyramine. Studies with several reversible MAO-A inhibitors, such as moclobemide and brofaromine, have indeed shown that their ability to cause a cheese reaction can be considerably lower than that observed with an irreversible inhibitor.

Studies on the polymorphisms of the MAO-A gene have indicated that a functional repeat polymorphism in the MAO-A gene promoter, with the longer alleles that were more active, is considerably more common in females with associated with panic and major depressive disorders. Indeed, it appears to be a risk factor for these conditions in females (Schulze et al., 2000).

6.2. Parkinson's disease

The neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which gives rise to a condition resembling idiopathic Parkinson's disease, is converted to the active toxin MPP^+ (1-methyl-4-phenyl-pyridinium) in successive oxidation steps catalyzed by MAO-B in glial cells. The protection against MPTP neurotoxicity afforded by MAO-B inhibitors has led to the suggestion that an endogenous or environmental toxin resembling MPTP may be responsible for idiopathic Parkinson's disease. However such a toxin has yet to be identified. Because cigarette smoking results in inhibition of MAO-B, it has been suggested that this may underlie the decreased incidence of Parkinson's disease in smokers.

Inhibitors of MAO might be expected to prolong the actions of dopamine in Parkinson's disease, and several studies have indicated that (-)-deprenyl potentiates the antiakinetic effect of L-dopa and may increase the life expectancy of patients treated with L-dopa and a peripheral inhibitor of dopa decarboxylase (Mardopar). In addition, treatment with (-)-deprenyl alone has been shown to increase the time between the initial diagnosis of Parkinson's disease and the necessity of commencing treatment with L-dopa. The results of prospective studies on the possible effects on overall life expectancy are awaited.

These results have led to the suggestion that (-)-deprenyl may have a neuroprotective effect, and evidence has been presented that it protects or rescues nerves from degeneration arising from a variety of chemical and physical insults by mechanisms distinct from its MAO-B inhibitory action. Some other acetylenic MAO inhibitors, including rasagiline, have been shown to act similarly, but others, such as clorgyline and pargyline, are less effective. In model systems, concentrations of (-)-deprenyl below those necessary to inhibit MAO have been reported to be neuroprotective or neuroescuing. These low effective concentrations should be born in mind when considering claims that the protective effects may be a result of an antioxidant action of such compounds. A convincing demonstration that the protective or rescuing effects were not dependent on MAO inhibition arose from the demonstration that dibenzo*[b,f]*oxepin-10-ylmethyl-methyl-prop-2-ynyl-amine (CGP 3466), which is structurally related to deprenyl but is ineffective as a MAO inhibitor, was 100 times more effective in neuroprotection (Kragten et al., 1998). Similarly, the *S*-isomer of rasagiline [R(+)-N-propargyl-1-aminioindan] has been shown not to inhibit MAO but, nevertheless, to resemble rasagiline in its neuroprotective behavior (Youdim et al., 2001)

Since the neuroprotective, or neurorescuing, actions of these compounds appear to be independent of their action as MAO inhibitors, further discussion of the possible mechanisms involved are outside the scope of this article. However, the possibility that inhibition of MAO may be a beneficial addition in the treatment of patients with Parkinson's disease has yet to be tested, although there are still arguments about whether the effects of (-)-deprenyl in human subjects are, essentially, symptomatic.

6.3. Alzheimer's disease

Significant increases in the MAO-B activities in hippocampus, cortex gyrus cinguli, and blood platelets have been reported in patients with dementia of the Alzheimer's type, as compared to control patients matched by age and sex. The selective increase of MAO-B activity in brain tissue might be explained by a higher neuronal loss and accompanying proliferation of extraneuronal tissue in this disease. However, the cause for the increase in platelet MAO activity is still a matter for speculation. Although it has been reported that no difference is found between in MAO-A activity in patients with Alzheimer's disease and control patients, the levels of mRNA for that enzyme, as well as for MAO-B, are elevated in the frontal cortex in the disease (Emilsson et al., 2002). These results suggested that selective MAO-B inhibitors may be beneficial in the treatment of this disease. Indeed (-)-deprenyl has been found to improve episodic memory and learning tasks requiring complex information processing in patients with dementia of the Alzheimer's type. Aspects of Alzheimer's disease are known to be ameliorated by acetylcholinestease (AchE) inhibitors; recently an analogue of the drug rasagiline that also bears a carbamate, AchE inhibitory, group has been developed for the possible therapy of this disease. This compound (TV3326) has been shown to exert AchE- and MAO-inhibitory activities in vivo and also to retain the neuroprotective actions of rasagiline. It also exhibits antidepressant activity in animal models (Youdim and Weinstock, 2002) Because, in common with other neurodegenerative diseases, evidence shows that oxidative stress may play a substantial role in the progress of Alzheimer's disease and the action of MAO may contribute to this, an alternative approach is to use a selective MAO-B inhibitor, such as lazabemide (Mason et al., 2000), which is also as an antioxidant.

6.4. Down syndrome

The activity of MAO-B in blood platelets was found to be significantly lower in subjects with Down syndrome as compared to normal control patients. However, this appears to be an age-dependent phenomenon. Administration of classical and nonselective inhibitors, such as nialamide, to patients with Down syndrome has been reported to produce increased motor activity and quickened mental and physical reactions but to give no convincing improvement in intellectual performance.

6.5. Norrie disease

This is a rare X-linked recessive neurologic disorder that involves a deletion of a segment of the X-chromosome. In the "classical" form this disorder is characterized by a retinal dysplasia and consequent congenital blindness. About half of the patients also manifest mental retardation, often with developmental regression or psychotic features appearing in early childhood and a progressive sensorineural hearing loss usually not evident until the second decade of life. The activities of MAO-A and the levels of its mRNA in cultured skin fibroblasts as well as the activities of MAO-B in the blood platelets do not appear to be affected in this disease. In contrast, an "atypical" form of this disease exists in which the deletion in the X-chromosome also affects the genes for MAO. Such patients have no measurable MAO-A or -B activity in the skin fibroblasts or platelets, respectively, and no detectable mRNA for MAO-A in their fibroblasts. The urinary and plasma amine and amine metabolites have been quantified in two individuals with "atypical" Norrie disease. A marked 4- to 100-fold elevation in levels of urinary PEA, *o*-tyramine and *m*-tyramine and marked reductions in levels of 3-methoxy-4-hydroxyphenylglycol in both urine and plasma confirmed a systemic, and functionally
significant, loss of the activities of both MAO-A and -B. The symptoms of "atypical" patients, in addition to those described for "classic" Norrie disease, include somatic growth failure, microcephaly, delayed sexual maturation, possible altered peripheral autonomic function, disordered sleep patterns, as well as profound mental retardation, myoclonus, and seizures. The studies with "atypical" patients indicate that while apparently complete loss of both MAO-A and -B activities is compatible with human life, it does cause a severe neurological phenotype

6.6. Attention-deficit hyperactivity disorder (ADHD)

Monoamine oxidase inhibitors have been shown to be effective in reducing or controlling patient symptoms in ADHD. There appears to be a genetic link between such disorders and the DXS7 locus on the X chromosome, and this locus is closely linked with the MAO genes (Jiang et al., 2000). The long promotor polymorphism in the MAO-A gene has also been reported to be an hereditary risk factor for this condition in both males and females (Manor et al., 2002).

6.7. Personality

The activity of blood platelet MAO-B is apparently bimodally distributed in the general population, and several studies have demonstrated a relationship between this and personality. It appears that low platelet MAO-B activity is associated with a "sensation-seeking" or "monotony-avoiding" personality, a group that tends to include a higher proportion of active suicides, mountaineers, aviators, and substance abusers. This may also underlie the reports that low platelet MAO-B activity is associated with alcoholism, although such a correlation has not been found in all studies, even when those with a family history of alcoholism were considered separately. Cigarette smoking is more associated with the "sensation-seeking" proband, and because, as discussed above, components of cigarette smoke inhibit MAO, it has been argued that this may account for the low platelet MAO-B observed in that group. However, it appears that cigarette smoking may account for only part of the association (Oreland et al., 2002).

6.8. Aggression

A condition in which a point mutation in exon 8 of the MAO-A gene resulted in complete deficiency of MAO-A activity was shown to result in a compulsively aggressive behavior in human males (Brunner et al., 1993). The types of behavior that occurred include impulsive aggression, arson, attempted rape, and exhibitionism. Similar behavioral abnormalities have been produced by "knocking out" the MAO gene in mice. The involvement of 5-HT in this behavior was shown by its abolition by the 5-HT_{2A} receptor antagonist ketanserin and by tetrabenazine, which is an inhibitor of the vesicular monoamine transporter (VMAT2). Because high levels of monoamine oxidase inhibition in the adult do not produce such dramatic effects, it is likely that the effects of gene deletion reflect, at least in part, developmental alterations. In contrast to the effects of MAO-A deletion, MAO-B knock-out mice showed no increase in aggressive behavior and relatively minor changes in behavioral tests, although they did show a reduced blood pressure in the resting state. However, several studies have reported reduced platelet MAO-B to be associated with criminal behavior

7. See Also:

Antidepressants
Psychiatry, biological
Mood disorders

8. Further reading

Abell CW, Kwan SW (2001): Molecular characterization of monoamine oxidases A and B. *Prog Nucleic Acid Res Mol Biol* 65:129-156

Damberg M, Garpenstrand H, Hallman J, Oreland L (2001) Genetic mechanisms of behavior-don't forget about the transcription factors *Mol Psychiatry* 6:503-510

Edmondson DE, Newton-Vinson P (2001): The covalent FAD of monoamine oxidase: Structural and functional role and mechanism of the flavinylation reaction. *Antioxid Redox Signal* 3:789-806

Kitani K, Minami C, Isobe K-I, Maehara K, et al. (2002): Why (-) deprenyl prolongs survivals of experimental animals: Increase of anti-oxidant enzymes in brain and other body tissues as well as mobilization of various humoral factors may lead to systemic anti-aging effects. *Mech Ageing Dev* 123:1087-1100

Maruyama W, Akao Y, Carrillo MC, Kitani K, et al. (2002): Neuroprotection by propargylamines in Parkinson's disease: Suppression of apoptosis and induction of prosurvival genes. *Neurotoxicol Teratol* 24:675-682

Shih JC, Chen K (1999): MAO-A and -B gene knock-out mice exhibit distinctly different behavior. *Neurobiology* 7:235-246

Tatton WG, Chalmers-Redman RM (1996): Modulation of gene expression rather than monoamine oxidase inhibition: (-)-deprenyl-related compounds in controlling neurodegeneration. *Neurology* 47:S171-S183

Tipton KF, Davey GP, Motherway M (2000): Monoamine oxidase assays. In: *Current Protocols in Pharmacology*, Taylor GP and Sullivan JP, eds. New York: Wiley, pp. 3.6.1-3.6.42

Tipton KF, Strolin Benedetti M (2001): Amine oxidases and the metabolism of xenobiotics. In: *Enzyme Systems that Metabolise Drugs and Other Xenobiotics*, Ioannides C, ed. Chichester: Wiley, pp.95-146

9. References

Arai R, Karasawa N, Kurokawa K, Kanai, H, et al. (2002): Differential subcellular location of mitochondria in rat serotonergic neurons depends on the presence and the absence of monoamine oxidase type B. *Neuroscience* 114:825-835 [MEDLINE]

Berlin I, Spreux-Varoquaux O, Launay JM (2000): Platelet monoamine oxidase B activity is inversely associated with plasma cotinine concentration. *Nicotine Tob Res* 2:343-346 [MEDLINE]

Binda C, Newton-Vinson P, Hubálek F, Edmondson DE, et al. (2002): Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nature Struct Biol* 9:22-26 [MEDLINE]

Brunner HG, Nelen M, Breakefield XO, Ropers HH, et al. (1993): Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* 262:578-580 [MEDLINE]

Emilsson L, Saetre P, Balciuniene J, Castensson A, et al. (2002): Increased monoamine oxidase messenger RNA expression levels in frontal cortex of Alzheimer's disease patients. *Neurosci Lett* 326:56-60 [MEDLINE]

Fornai F, Chen K, Giorgi FS, Gesi M, et al. (1999): Striatal dopamine metabolism in monoamine oxidase B-deficient mice: A brain dialysis

study. *J Neurochem* 73:2434-2440 [MEDLINE]

Fowler JS, Ding YS, Logan Y, MacGreggor RR, et al. (2001): Species differences in [11C]clorgyline binding in brain. *Nucl Med Biol* 28:779-785 [MEDLINE]

Jiang S, Xin R, Lin S, Qian Y, et al. (2000): Linkage studies between attention-deficit hyperactivity disorder and the monoamine oxidase genes. *Am J Med Genet* 105:783-788

Kragten E, Lalande I, Zimmermann K, Roggo S, et al. (1998): Glyceraldehyde-3-phosphate dehydrogenase, the putative target of the antiapoptotic compounds CGP 3466 and R-(-)-deprenyl. *J Biol Chem* 273:5821-5828 [MEDLINE]

Krueger MJ, Mazouz F, Ramsay RR, Milcent R, et al. (1995): Dramatic species differences in the susceptibility of monoamine oxidase B to a group of powerful inhibitors. *Biochem Biophys Res Commun* 206:556-562 [MEDLINE]

Manor I, Tyano S, Mel E, Eisenberg J, et al. (2002): Family-based and association studies of monoamine oxidase A and attention deficit hyperactivity disorder (ADHD): Preferential transmission of the long promoter-region repeat and its association with impaired performance on a continuous performance test (TOVA). *Mol Psychiatry* 6:626-632 [MEDLINE]

Mason RP, Olmstead EG, Jacob RF (2000): Antioxidant activity of the monoamine oxidase B inhibitor lazabemide. *Biochem Pharmacol* 60:709-716 [MEDLINE]

Miller JR, Edmondson DE (1999): Structure-activity relationships in the oxidation of para-substituted benzylamine analogues by recombinant human liver monoamine oxidase A. *Biochemistry* 41:13670-83 [MEDLINE]

O'Brien EM, Dostert P, Tipton KF (1995): Species differences in the interactions of the anticonvulsant milacemide and some analogues with monoamine oxidase-B. *Biochem Pharmacol* 50:317-324 [MEDLINE]

Oreland L, Damberg M, Hallman J, Garpenstrand H (2002): Smoking only explains part of the associations between platelet monoamine oxidase activity and personality. *J Neural Transm* 199:963-975

Rebsam A, Seif I, Gaspar P (2002): Refinement of thalamocortical arbors and emergence of barrel domains in the primary somatosensory cortex: a study of normal and monoamine oxidase A knock-out mice. *J Neurosci* 22:8541-8552 [MEDLINE]

Schulze TG, Muller DJ, Krauss H, Scherk H, et al. (2000): Association between a functional polymorphism in the monoamine oxidase A

gene promoter and major depressive disorder. *Am J Med Genet* 96:801-803

Shih JC, Ridd MJ, Chen K, Meehan WP, et al. (1999): Ketanserin and tetrabenazine abolish aggression in mice lacking monoamine oxidase A. *Brain Res* 835:104-112 [MEDLINE]

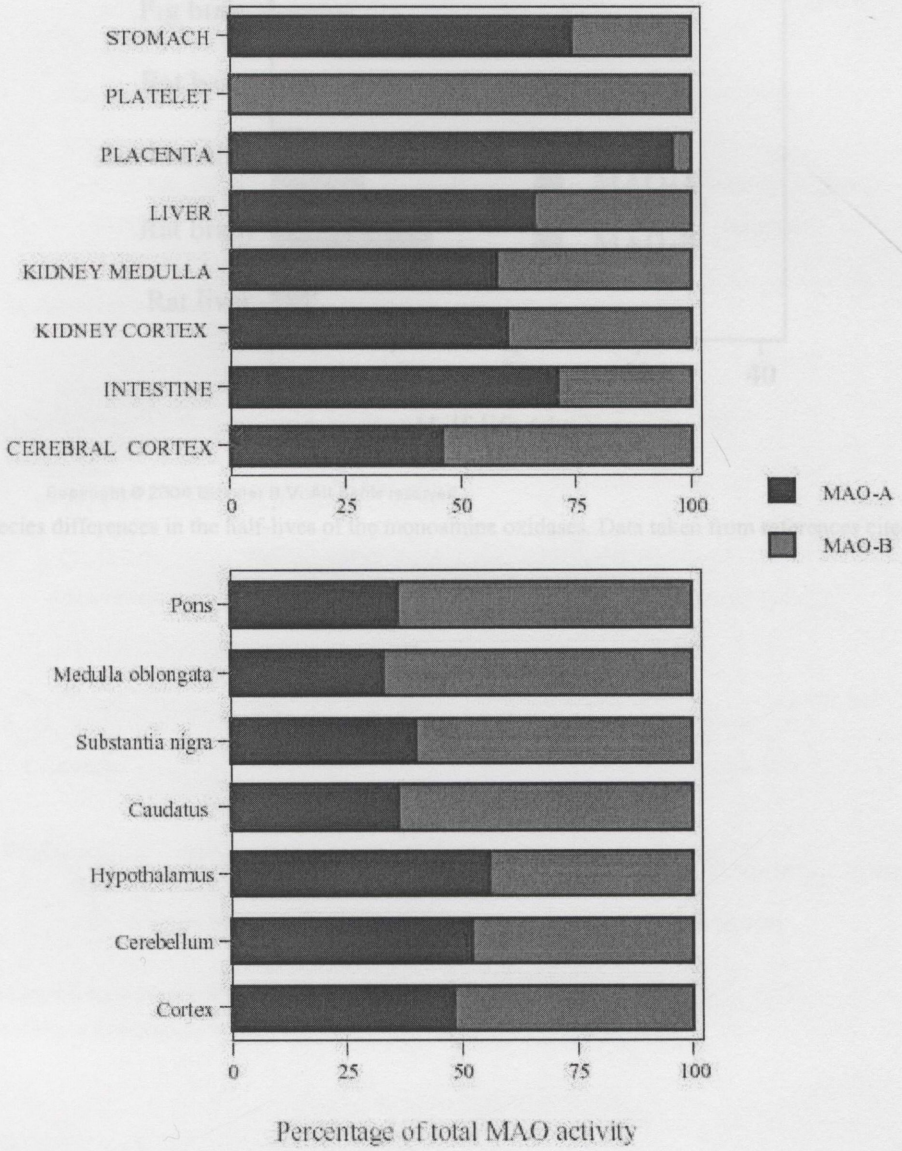
Suarez-Merino B, Bye J, McDowall J, Ross M, et al. (2001): Sequence analysis and transcript identification within 1.5 MB of DNA deleted together with the NDP and MAO genes in atypical Norrie disease patients presenting with a profound phenotype. *Hum Mutat* 17:523

Wong WK, Ou X-M, Chen K, Shih, JC (2002): Activation of human monoamine oxidase b gene expression by a protein kinase C MAPK signal transduction pathway involves c-Jun and Egr-1. *J Biol Chem* 277:22222-22230 [MEDLINE]

Youdim MBH, Weinstock M (2002): Novel neuroprotective anti-Alzheimer drugs with anti-depressant activity derived from the anti-Parkinson drug, rasagiline. *Mech Ageing Dev* 123:1081-1086 [MEDLINE]

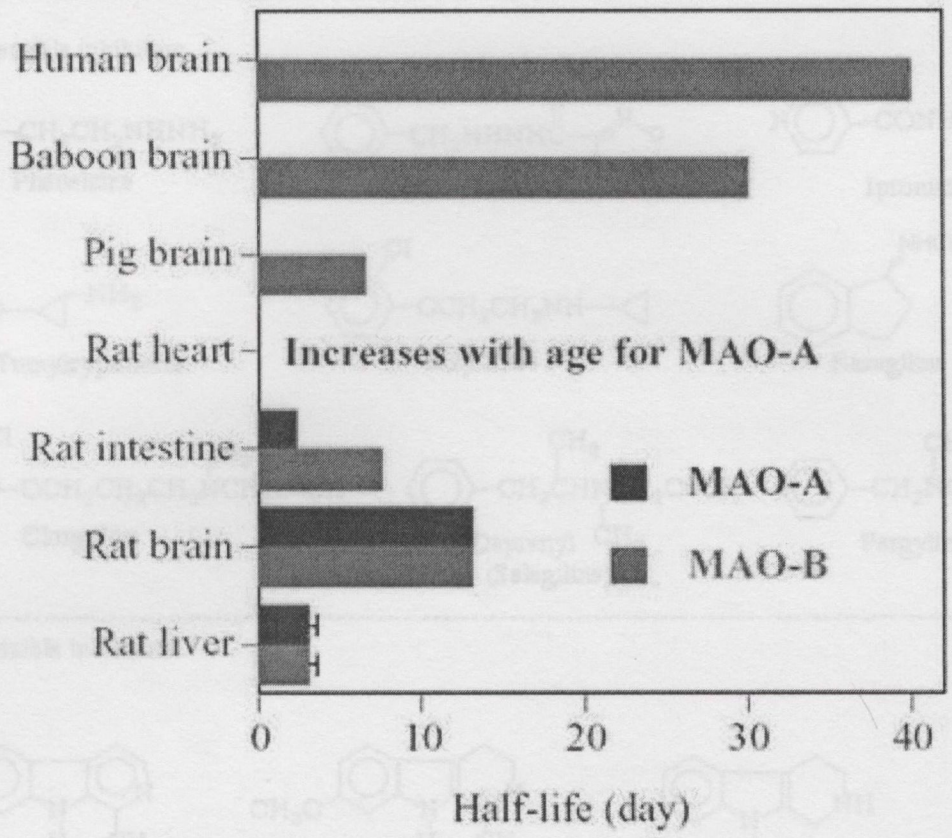
Youdim MBH, Wadia A, Tatton W, Weinstock M (2001) The anti-Parkinson drug rasagiline and its cholinesterase inhibitor derivatives exert neuroprotection unrelated to MAO inhibition in cell culture and *in vivo*. *Ann N Y Acad Sci* 939:450-458

Fig.1 The relative contributions of MAO-A and -B to the total activity in different human tissues and brain regions. Activities in shown in the upper panel were determined towards tyramine (100 μM) and those in the different brain regions were determined towards dopamine.



Copyright © 2004 Elsevier B.V. All rights reserved.

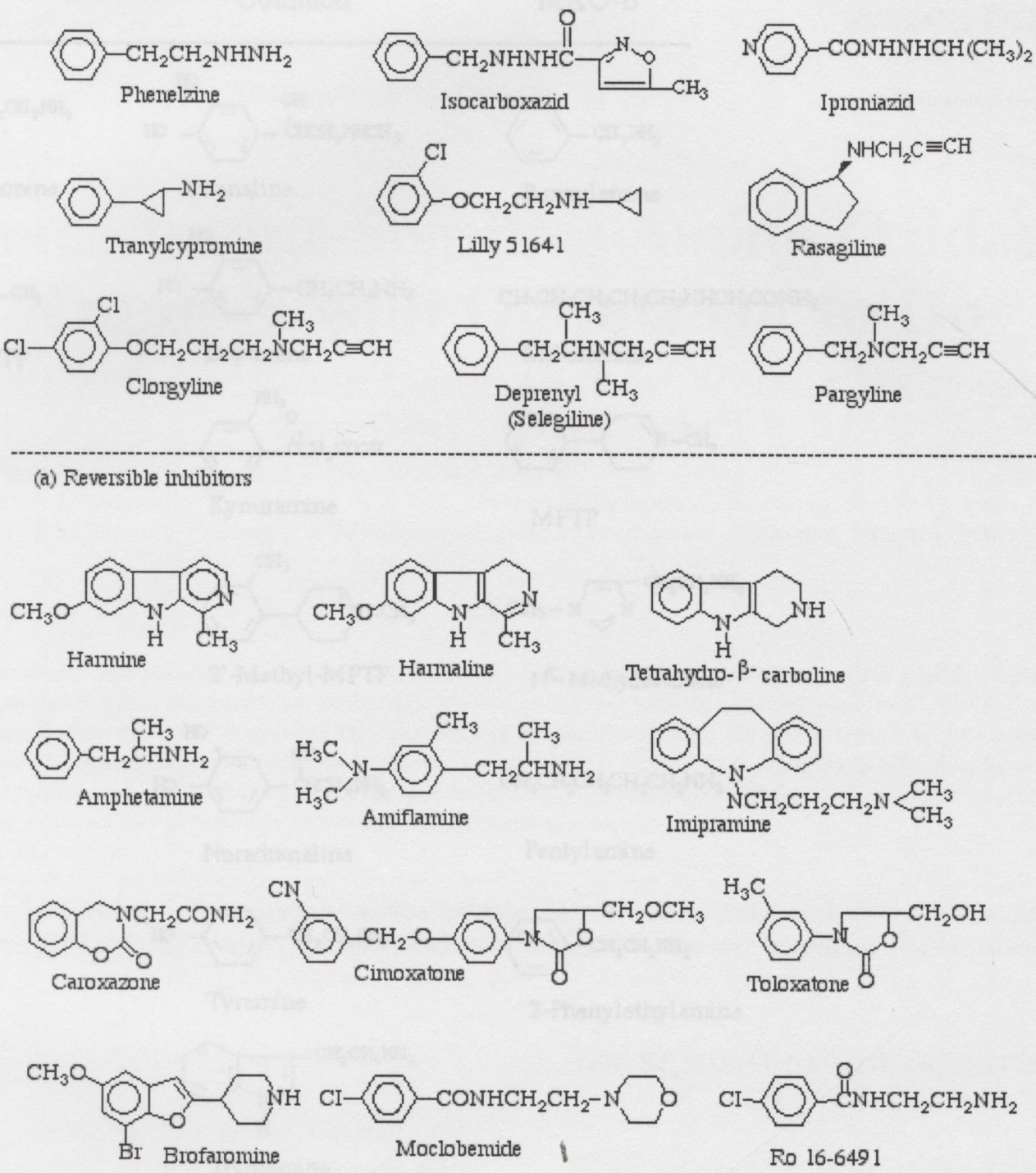
Figure 1 The relative contributions of MAO-A and -B to the total activity in different human tissues and brain regions. Activities in shown in the upper panel were determined towards tyramine (100 μM) and those in the different brain regions were determined towards dopamine.



Copyright © 2004 Elsevier B.V. All rights reserved.

Figure 2 Tissue and species differences in the half-lives of the monoamine oxidases. Data taken from references cited in the text

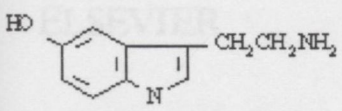
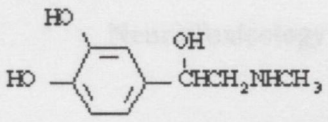
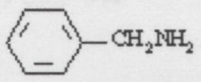
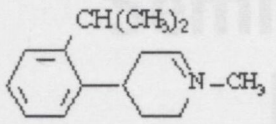
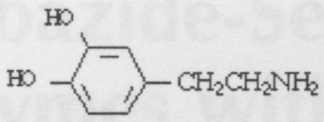
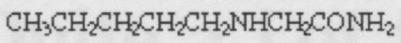
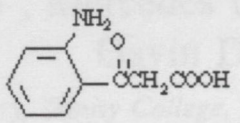
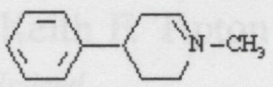
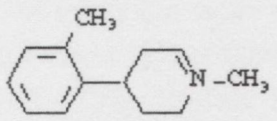
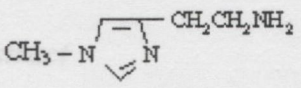
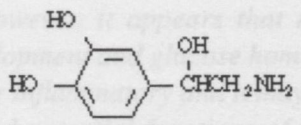
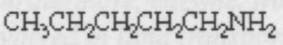
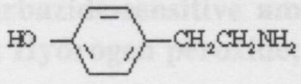
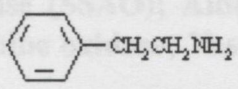
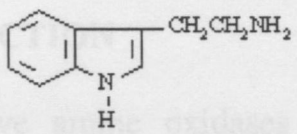
Fig. 3 Some MAO Inhibitors Referred to in the Text.



Copyright © 2004 Elsevier B.V. All rights reserved.

Figure 3 Some MAO Inhibitors Referred to in the Text.

Table 1 Substrate preferences of MAO-A and -B

MAO-A	Common	MAO-B
 5-Hydroxytryptamine	 Adrenaline	 Benzylamine
 2'-Isopropyl-MPTP	 Dopamine	 Milacemide
	 Kynuramine	 MPTP
	 2'-Methyl-MPTP	 N^{τ}-Methylhistamine
	 Noradrenaline	 Pentylamine
 Tyramine	 2-Phenylethylamine	
 Tryptamine		

SCIENCE DIRECT

SCIRUS
for scientific information only

Copyright © 2004 Elsevier B.V. All rights reserved.

ELSEVIER

NeuroToxicology 25 (2004) 303–315

NeuroToxicology

Semicarbazide-Sensitive Amine Oxidases: Enzymes with Quite a Lot to Do

Jeff O'Sullivan[1], Mercedes Unzeta[2], Joe Healy[1], Michael I. O'Sullivan[3], Gavin Davey[1], Keith F. Tipton[1,*]

[1]*Department of Biochemistry, Trinity College, Dublin 2, Ireland*
[2]*Department de Bioquimica i de Biologia Molecular, Universitat Autonoma de Barcelona, Barcelona, Spain*
[3]*Department of Restorative Dentistry & Periodontology, Dublin Dental School & Hospital, Dublin 2, Ireland*

Abstract

The semicarbazide-sensitive amine oxidases (SSAO) (EC 1.4.3.6) were believed to be detoxifying enzymes, primarily involved in the oxidative deamination of endogenous amines, such as methylamine and aminoacetone, together with some xenobiotic amines. However, it appears that the reaction products may have important signalling functions in the regulation of cell development and glucose homeostasis. Furthermore, enzyme, from some sources, behaves as a cellular adhesion protein under inflammatory and it may also be involved in lipid transport. This review considers what is known about the activities and potential functions of this hardworking protein.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Semicarbazide-sensitive amine oxidase (SSAO); Aldehyde; Amiloride-binding protein; Diabetes; GLUT-4; Hydrogen peroxide; Monoamine oxidase; Vascular adhesion; VAP-1; Xenobiotic

INTRODUCTION

The semicarbazide-sensitive amine oxidases (EC 1.4.3.6: amine:oxygen oxidoreductase (deaminating) (copper-containing); SSAOs) have long been the poor relations of the monoamine oxidases (EC 1.4.3.4: amine:oxygen oxidoreductase (deaminating) (flavin-containing); MAO). Their physiological functions were unclear and, unlike the monoamine oxidases, there were no specific inhibitors with identified therapeutic applications (see Callingham et al., 1995; Houen, 1999; Klinman and Mu, 1994; Lewinsohn, 1984; Lyles, 1995, 1996; Tipton et al., 2000 for reviews on different aspects of these enzymes). However, more recently, it has become apparent that SSAO may have diverse biological roles. This article will, briefly review the properties and behaviour of SSAO before considering the complexities of its possible biological functions.

Abbreviations: SSAO, semicarbazide-sensitive amine oxidase; MAO, monoamine oxidase; TNF, tumour necrosis factor; VAP, vascular adhesion protein

* Corresponding author.

THE SEMICARBAZIDE-SENSITIVE AMINE OXIDASE FAMILY

SSAO catalyses the oxidative deamination of primary amines, according to the overall reaction

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + NH_3 + H_2O_2$$

It is a rather diverse group of enzymes which also appear to differ significantly between tissues and species. Their sensitivity to inhibition by carbonyl-group reagents, such as semicarbazide, results from the presence of a TOPA (6-hydroxydopa; 2,4,5-trihydroxyphenylalanine) quinone residue as the redox cofactor (see Janes and Klinman, 1995; Houen, 1999). This distinguishes it from the monoamine oxidases (EC 1.4.3.4: amine:oxygen oxidoreductase (deaminating) (flavin-containing)) which are not inhibited by semicarbazide. Conversely, SSAO is insensitive to inhibition by the acetylenic monoamine oxidase inhibitors, such as clorgyline, l-deprenyl and pargyline. Because of this SSAO was sometimes referred to as clorgyline-resistant amine oxidase (CRAO).

The name SSAO is not particularly helpful, since some other amine oxidases are also inhibited by semi-

0161-813X/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0161-813X(03)00117-7

carbazide. These include diamine oxidase (DAO) (also classified as EC 1.4.3.6) and lysyl oxidase (see Smith-Mungo and Kagan, 1988), which differs from the other members of this group in having lysine tyrosylquinone rather than TOPA-quinone as cofactor (Wang et al., 1997). Since the specificities of DAO, which is found in high levels in the intestine, kidney, thymus gland and placenta (see Buffoni, 1966), and SSAO overlap, it is not always clear from publications which enzyme was being studied. By usage, SSAO has become defined by a negative: the semicarbazide-sensitive amine oxidase that has little activity towards diamines such as histamine and other diamines, putrescine, cadaverine, spermidine and spermine, which are DAO substrates.

In most mammals, the enzyme exists in tissue-bound and soluble (plasma) forms, but there are wide species and tissue differences in the activities (Boomsma et al., 2000a, 2000b). Homology-modelling studies suggest that tissue-bound contains a short intracellular domain, a single transmembrane domain and a long extracellular domain that includes the catalytic site (Salminen et al., 1998). Plasma SSAO appears to be the result of proteolytic cleavage of membrane-bound SSAO and it has been suggested that the primary source for the plasma SSAO is the liver (Kurkijärvi et al., 1998). However, Ekblom et al. (2000) have provided evidence that at least some plasma SSAO may be derived from bone tissue. Furthermore, it appears that there may be more than one SSAO enzyme in plasma from some species (see, e.g. Boomsma et al., 2000a, 2000b; Elliott et al., 1992). In ruminants at least one of these additional enzymes may correspond to the plasma polyamine oxidase, which deaminates the polyamines spermine and spermidine at the primary amino group (see Morgan, 1985).

SSAO is present, in varying amounts in many mammalian tissues (see Andrés et al., 2001). Relatively high activities of SSAO are associated cardiovascular smooth muscle cells, adipose tissue, lung and cartilage. It is absent from the nerves and glial cells of the brain, but present in the microvessels of brain (Castillo et al., 1999) and, thus may contribute to the 'blood–brain barrier'. Although absent from nerves in the CNS, it appears that the enzyme may be associated with the nerves in dental pulp (O'Sullivan et al., 2002).

The mammalian enzyme (Mr 180,000) is a dimeric, heavily glycosylated, protein that contains 1 mol of copper per subunit (see Knowles and Yadav, 1984). This copper ion, Cu(II), appears to be essential for the double hydroxylation of a tyrosine residue in the polypeptide chain of SSAO, by an autocatalytic reaction that yields the TOPA cofactor (Cai et al., 1997). Although copper centres are involved in a number of oxidase reactions, it appears that its role in amine oxidation by SSAO is restricted to providing a suitably placed positive charge in the active site (see McGuirl and Dooley, 1999).

6-Hydroxydopamine is a well-known catecholaminergic neurotoxin (see, e.g. Cadet and Brannock, 1998), which has been reported to be formed in vivo (Andrew et al., 1993). However, there are no data to indicate whether the 6-hydroxydopa that would be formed as a result of SSAO degradation in vivo contributes significantly, after decarboxylation, to the endogenous levels of this compound.

It appears that mammals contain two genes encoding human SSAO (Imamura et al., 1997; Zhang and McIntire, 1996), plus a pseudo-gene (Cronin et al., 1998). One of these encodes the SSAO present in most tissues whereas the other codes for a form of SSAO that has, so far, only been found in the retina (Imamura et al., 1997). Unfortunately, little is known about the behaviour or function of retinal SSAO whose primary structure has rather low (64%) sequence identity to SSAO from other mammalian tissues.

SUBSTRATES

Some SSAO substrates are listed in Fig. 1. There are large species differences in the specificities of SSAO, but the non-physiological amine benzylamine is a good substrate for the mammalian enzymes. Indeed, plasma SSAO has sometimes been referred to as benzylamine oxidase. The physiological substrates are believed to include aminoacetone (Lyles and Chalmers, 1992), methylamine (Precious et al., 1988), 2-phenylethylamine, tyramine (Young et al., 1982) and dopamine (Lizcano et al., 1991a, 1991b). Many of the substrates for SSAO are also oxidatively deaminated by MAO, but aminoacetone and methylamine are not MAO substrates. Although 5-HT is not a substrate for SSAO from most sources, it is a good substrate for the enzyme from pig and human dental pulp (Nordqvist et al., 1982; O'Sullivan et al., 2003a, 2003b). SSAO also catalyses the oxidative deamination of a number of xenobiotics (see Tipton and Strolin Benedetti, 2001), including mescaline and the anti-malarial drug, primaquine.

The fact that the active site of the tissue-bound SSAO appears to be located in the extracellular domain (see McGuirl and Dooley, 1999; Salminen et al., 1998), would be consistent with its being involved, together with the plasma enzyme, in the inactivation of potentially toxic amines in the blood. In contrast, the monoamine oxidases are intracellullar enzymes that are associated with the mitochondrial outer membrane.

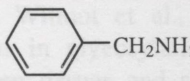
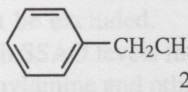
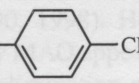
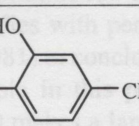
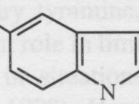
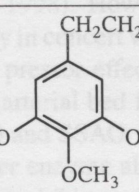
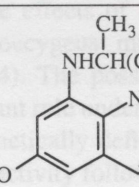
SUBSTRATES	COMMENTS
CH_3NH_2 Methylamine	Endogenous and xenobiotic. Not a substrate for MAO
$CH_2{=}CHCH_2NH_2$ Allylamine	Xenobiotic. Not a substrate for MAO. Highly toxic product
$CH_3{-}\overset{\overset{O}{\|}}{C}{-}CH_2NH_2$ Aminoacetone	Endogenous. Not a substrate for MAO
$CH_3(CH_2)_3CH_2NH_2$ *n*-Pentylamine	Xenobiotic. Also MAO-B substrate
 CH_2NH_2 Benzylamine	Xenobiotic Also MAO-B substrate
 $CH_2CH_2NH_2$ 2-Phenethylamine	Trace amine Also MAO-B substrate
HO  $CH_2CH_2NH_2$ Tyramine	Endogenous & xenobiotic Also MAO A & B substrate
HO HO  $CH_2CH_2NH_2$ Dopamine	Endogenous Also MAO A & B substrate
HO  $CH_2CH_2NH_2$ 5-Hydroxytryptamine	Substrate in dental pulp. MAO-A substrate
$CH_2CH_2NH_2$  Mescaline	Xenobiotic. Also MAO substrate
CH_3 $NHCH(CH_2)_3NH_2$  Primaquine	Xenobiotic. Also MAO substrate

Fig. 1. Some compounds that have been shown to be SSAO substrates.

However, the involvement of SSAO in the metabolism of amines that are also substrates for MAO has not been easy to establish because wide species differences in specificities and amount of enzyme present. For example, high SSAO activities are found in pig and sheep plasma, the levels are very much lower in human plasma and it is often difficult to detect any at all in the rat. The levels of the tissue-bound enzyme also vary widely between species but do not parallel those of the plasma enzyme (see Boomsma et al., 2000a, 2000b). Differences in substrate specificities between the SSAO enzymes from different mammalian sources also complicate the situation. For example, mescaline has been shown to be oxidised more efficiently than benzylamine by pig plasma SSAO (Buffoni and Della Corte, 1972) but the human enzyme has no detectable activity towards this substrate. Such differences also appear to extend to stereospecificity, since oxidation of benzylamine by plasma SSAO from ox, horse, porcine, rabbit, and sheep involves abstraction of the pro-*S* hydrogen (Alton et al., 1995) whereas SSAO from human aorta and plasma has been reported to show no absolute stereospecificity in this respect (see Yu and Davis, 1988). Thus, attempts to extrapolate from the

situation in experimental animals to that in the human should be interpreted with great caution.

The three-dimensional structure of mammalian SSAO has yet to be determined. However, resolution of the crystal structures of the copper-containing amine oxidases from several other sources has revealed considerable differences in active-site accessibility that may account for specificity differences (see McGuirl and Dooley, 1999; Wilmot et al., 1997). Possible effects of variations in glycosylation, which differ considerably between tissues and species (see Holt et al., 1998), cannot be excluded.

The relatively high SSAO levels in lung may protect against inhaled methylamine and other volatile amines (see Lizcano et al., 1990, 1998). However, although oxidative deamination by MAO appears to play a major role in the presystemic elimination of amines such as 2-phenylethylamine, studies with perfused rabbit lung led Gewitz and Gillis (1981) to conclude that SSAO did not play a significant role in this process. Similarly, although intestinal MAO makes a large contribution to the metabolism of dietary tyramine, intestinal SSAO does not play a significant role in limiting the effects of dietary tyramine, even in situations where MAO is inhibited (Hasan et al., 1998). However, SSAO does appear to work effectively in concert with MAO in some tissue preparations. The pressor effects of tyramine in the perfused mesenteric arterial bed from the rat, were potentiated if both MAO and SSAO were inhibited but not by inhibition of either enzyme alone (Elliott et al., 1989b). In contrast, SSAO inhibitors alone were found to potentiate the contractile effects of sympathomimetic amines on a rat anococcygeus muscle preparation (Callingham et al., 1984). The possibility that SSAO may play a more important role under conditions where MAO is inhibited or genetically deficient is suggested by a transient rise in its activity following MAO inhibition in vivo (Fitzgerald and Tipton, 2002).

Another enzyme that may compete with SSAO, particularly in blood plasma is ceruloplasmin (EC 1.16.3.1). This is a blue coloured plasma protein that binds up to 95% of circulating copper. Although copper binding and transport may be its main function, it also catalyses amine oxidation; mediating a four-electron reduction of oxygen by substrate without releasing O_2^- or H_2O_2 as intermediates (Calabrese et al., 1989). It has a relatively broad oxidase specificity for organic molecules and has the ability to reduce O_2 to H_2O in successive one-electron steps. Physiologically important substrates include DOPA, noradrenaline, adrenaline and serotonin (Rydén, 1984). It is most active at low pH values and shows no significant activity towards benzylamine. The protein is also expressed in liver, which is believed to be the origin of circulating ceruloplasmin, and other tissues, with lung being a relatively rich source (see Yang et al., 1996). There is no evidence that it contributes significantly to the oxidation of SSAO substrates in plasma or elsewhere.

INHIBITORS

The lack of, readily available, selective inhibitors of SSAO, also hampers attempts to define its functions. Semicarbazide and cyanide are both SSAO inhibitors that also inhibit a number of other enzymes. The compound MDL 72145 ((*E*)-2-(3′,4′-dimethoxyphenyl)-3-fluoroallylamine), which has been shown to be a potent irreversible inhibitor of SSAO in rat aorta (Lyles and Fitzpatrick, 1985; Palfreyman et al., 1994) and brown adipose tissue (Elliott et al., 1989a), has been used in a number of studies on the metabolic role of SSAO. However, it is also a potent inhibitor of MAO-B and affects MAO-A activity to a lesser extent (see Zreika et al., 1984). Substituted β-chloroallylamines are, however, generally weak inhibitors of MAO and (*E*)-β-phenyl-3-chloroallylamine (MDL 72274) shows high potency and selectivity for SSAO in vitro, compared with its activity against MAO (Lyles et al., 1987; McDonald et al., 1985). 3,5-Diethoxy-4-aminomethylpyridine (also known as B24) appears to be a highly selective SSAO inhibitor, but is in fact a poor substrate for the enzyme (Buffoni et al., 1998). 2-Bromoethylamine was shown to be a potent and apparently selective mechanism-based inhibitor of SSAO (Kinemuchi et al., 2000), which also appears to be effective in vivo (Yu et al., 2001). Hydrazine derivatives are also SSAO inhibitors, but many of these are also inhibitors of MAO. The drug procarbazide (*N*-isopropyl-α-(2-methyl hydrazino)-*p*-toluamide hydrochloride) together with its metabolite monomethylhydrazine appears to be highly selective for SSAO both in vivo and in vitro (Holt and Callingham, 1995). However, it appears that there may be species and tissue differences in the sensitivities of SSAO to inhibition by hydrazine derivatives (Lizcano et al., 1996); again indicating the difficulty of extrapolating from results obtained in one species to the responses in another.

POTENTIALLY NASTY PRODUCTS

Perhaps because it has been so difficult to define metabolic functions for SSAO, much attention has

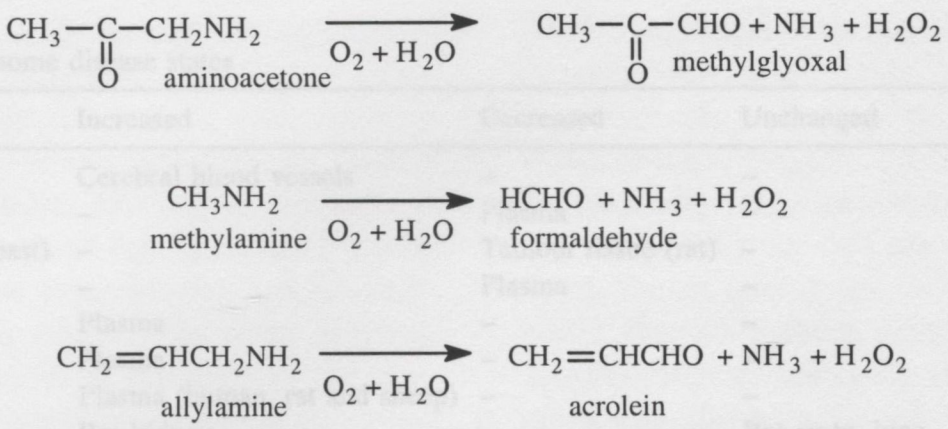


Fig. 2. Production of some potentially toxic aldehydes by SSAO.

been concentrated on the fact that the products of the SSAO catalysed reaction are potentially more toxic than the substrates that it oxidises. In overall terms the products, an aldehyde, ammonia and hydrogen peroxide, are the same as those produced in the MAO catalysed reaction. However, MAO produced its products within the cell whereas those formed by SSAO may be largely extracellular. Furthermore, the pathophysiological significance of the metabolite concentrations that have been used in some studies purporting to demonstrate metabolite toxicity, is unclear.

The aldehyde products of the SSAO reaction that have received most attention in terms of their potential toxicity are shown in Fig. 2. Aldehydes formed may be further oxidised to the corresponding carboxylic acid by aldehyde dehydrogenase or aldehyde oxidase or reduced to the corresponding alcohols by the aldehyde reductases or alcohol dehydrogenase. Formaldehyde produced by the oxidative deamination of methylamine is potentially toxic (see Heck et al., 1990). It is not a good substrate for any of these enzymes but may be rapidly metabolised by the glutathione-dependent formaldehyde dehydrogenase (EC 1.2.1.1) in mammalian tissues. Although the activity of this enzyme is high in most tissues, it is not present in the blood plasma, so any formaldehyde produced there would have to be transported into cells, such as the erythrocytes for metabolism. This may be significant with regarding formaldehyde-induced toxicity in blood vessels. Indeed, the in vivo toxicity of methylamine in the rat was shown to be reduced by the inhibition of SSAO (Yu, 1998). The consumption of relatively large amounts of creatine, in attempts to enhance performance in sports, would result in increased methylamine formation and, it has been suggested that the formation of formaldehyde and H_2O_2 from its oxidation might underlie some of the deleterious effects of long-term creatine consumption (Yu and Deng, 2000).

Metabolism of the xenobiotic allylamine by SSAO produces acrolein, which causes vascular toxicity. This can be prevented by inhibition of SSAO (see Conklin et al., 1998). It appears that this toxicity may result from the synergistic action of acrolein and hydrogen peroxide, since the presence of catalase reduced the extent of the damage caused by allylamine oxidation (Ramos et al., 1988).

The situation concerning methylglyoxal, formed by the SSAO-catalysed oxidation of aminoacetone, is less clear-cut. It is potentially toxic but it can It may be metabolised by other enzymes (see Thornalley, 1996) and, perhaps, also non-enzymically (Dutra et al., 2001). However, It does appear that SSAO may make a significant contribution to the process in vivo (Mathys et al., 2002).

SSAO AND DISEASE

The activity of SSAO appears to be altered in a number of disease states, as summarised in Table 1 (for review, see Lyles, 1996). Plasma SSAO activity is decreased in subjects suffering from severe burns but increased in cardiac disease (Boomsma et al., 2000a, 2000b) and in congestive heart failure it increases with the severity of the condition (Boomsma et al., 1997). SSAO activity is decreased in rat breast tumours (Lizcano et al., 1991a, 1991b) and SSAO expression is increased in cerebral blood vessels of subjects with Alzheimer's disease (Ferrer et al., 2002). Much attention has been devoted to the increase in plasma SSAO activity in diabetic patients (both types 1 and 2) (see Boomsma et al., 1995; Nilsson et al., 1968). Increased SSAO activity has also been observed in sheep plasma, rat plasma and kidney, in their respective experimental diabetic models but the activity is unchanged in rat lung, aorta and pancreas of diabetic rats. SSAO has also been shown to be decreased in white adipose tissue from, the diabetes-prone, obese Zucker rats (Moldes et al., 1999). The activity of the human plasma enzyme is further increased in complications arising

Table 1
Altered levels of SSAO in some disease states

Condition	Increased	Decreased	Unchanged	Reference
Alzheimer's disease	Cerebral blood vessels	–	–	Ferrer et al. (2002)
Burns	–	Plasma	–	See Lyles (1996)
Cancer (solid tumour in breast)	–	Tumour tissue (rat)	–	Lizcano et al. (1991a, 1991b)
Cancer (breast)	–	Plasma	–	See Lyles (1996)
Cardiac disease	Plasma	–	–	Boomsma et al. (2000a, 2000b)
Congestive heart failure	Plasma	–	–	Boomsma et al. (1997)
Diabetes (types 1 and 2)	Plasma (human, rat and sheep)	–	–	See Lyles (1996)
Diabetes	Rat kidney	–	Rat aorta, lung and pancreas	See Lyles (1996)
Diabetic retinopathy	Plasma	–	–	Gronvall-Nordquist et al. (2001)
Diabetic atherosclerosis	Plasma	–	–	Karádi et al. (2002)
Hypertension	–	–	Plasma	See Lyles (1996)
Inflammatory liver disease	Plasma	–	–	Kurkijärvi et al. (1998)
Kidney transplant rejection	Plasma	–	–	Kurkijärvi et al. (2001)
Pre-eclampsia[a]	–	–	Placenta	Sikkema et al. (2002)
Stroke	–	–	Plasma	Garpenstrand et al. (1999b)

Data refer to the human enzyme unless otherwise indicated.
[a] Maternal plasma SSAO activity has also been shown not to change significantly during normal human foetal growth (Lewinsohn and Sandler, 1982).

from diabetes, including diabetic retinopathy and atherosclerosis (Gronvall-Nordquist et al., 2001; Karádi et al., 2002). It has been suggested that increased methylglyoxal formation by the SSAO-catalysed oxidation of aminoacetone may contribute to the formation of advanced-glycation end products (AGEs), which are associated with vascular complications of diabetes (Mathys et al., 2002), although formaldehyde, from methylamine oxidation and increased hydrogen peroxide formation may also contribute (see, e.g. Garpenstrand et al., 1999a; Meszaros et al., 1999; Yu, 1998).

The mechanisms underlying these changes in SSAO activity are unclear and it appears that different processes may be involved. Increased plasma levels of the enzyme in cardiovascular disease may, at reflect release of the enzyme from dying cells and as discussed later, decreases in activity in some conditions may be connected with its vascular-adhesion function. A low molecular weight activator of SSAO has been found in human blood, the levels of which change in some disease states (Dalfó et al., 2003), but the increased cerebral blood vessel SSAO in subjects with Alzheimer's disease has been shown to reflect an increase in immunoreactive SSAO protein (Ferrer et al., 2002). There has also been a report of an endogenous SSAO inhibitor in human plasma (Buffoni et al., 1983) but this has not been characterised and there are no data on whether its levels vary under different pathophysiological conditions. The cytokine tumour necrosis factor-alpha (TNF-α) has been shown to decrease the SSAO mRNA and protein levels in pre-adipocyte cell line, as do effectors of the cyclic-AMP pathway (Mercier et al., 2003). However, the increased SSAO activity in adipose tissue of adipose tissue of mice made diabetic by treatment with alloxan was found to be accompanied by a decrease, rather than an increase in the SSAO-mRNA levels in this tissue (Nordquist et al., 2002).

The copper containing enzymes ceruloplasmin and DAO have been shown to support endothelial-cell mediated low-density lipoprotein (LDL) oxidation. This process appears to depend on the presence of copper but to be independent from the amine oxidase activity (Exner et al., 2001). Thus, these proteins may contribute, as pathophysiological catalyst of LDL modification, to atherogenic modification of vascular cells. However it is not known whether SSAO can act in this way.

ALTERNATIVE FUNCTIONS OF SSAO

Actions of the Products

Although they are potentially toxic at higher concentrations, the products of the SSAO-catalysed reactions may have important signalling roles at more physiological concentrations. Almost all the attention has been focused upon the potential role of hydrogen peroxide in this respect.

Insulin-like Actions

Although SSAO is largely associated with the cell membrane, a relatively small proportion has been

shown to be associated with the endosomal vesicles that contain the glucose transporter GLUT-4 in adipocytes. Insulin induces a recruitment of these intracellular GLUT-4 receptors to the cell-surface, thereby stimulating glucose uptake. Hydrogen peroxide is known to mimic the effects of insulin, although relatively high concentrations are necessary and the addition of vanadate is also required in some systems. Incubation of rat adipocytes in presence of the non-physiological SSAO substrate benzylamine and a low concentration of vanadate, was shown stimulate glucose transport, about six-fold. Both components are themselves unable to modify the basal glucose-transport rate. Several other SSAO substrates have been shown to have similar effects. Addition of semicarbazide abolished completely the effect, consistent with SSAO activity being involved. Furthermore, the activation of glucose transport was shown to be abolished by the presence of catalase (Enrique-Tarancon et al., 1998, 2000). Thus, it appears that the generation of H_2O_2 during the oxidation of substrates by SSAO stimulates glucose uptake and by the recruitment of insulin receptors to the cell-surface. Similar effects have been reported in cells derived from other insulin-sensitive tissues and the stimulation of glucose uptake has been shown to be accompanied by an antilipolytic effect (see Morin et al., 2001).

Administration of benzylamine plus vanadate, both acutely and chronically, has been shown to improve glucose tolerance in rats that had been rendered diabetic by treatment with streptozotocin. These in vivo antidiabetic effects were not seen when either vanadate or benzylamine were administered alone and did not involve significant changes in plasma insulin concentrations (Marti et al., 2001). It is believed that the H_2O_2 formed reacts with vanadate to form peroxyvanadate, which is the active effector in such systems. However, the necessity for vanadate casts doubt on the physiological significance of such models. However, in cultured human adipocytes (Morin et al., 2001) and rat aortic smooth muscle cells (El Hadri et al., 2002) SSAO substrates have been shown to stimulate glucose uptake in the absence of vanadate. The ability to stimulate glucose uptake is not a specific function of SSAO, since the hydrogen peroxide formed in the MAO-catalysed reaction can do the same, but the association of a proportion of SSAO in adipocytes with the intracellular vesicles containing the GLUT4 glucose transporter, which may suggest a more specific function for this enzyme. In this context, it is interesting that downregulation of SSAO expression by treatment of mice with TNF-α which is synthesised in fat cells and known to be involved in obesity-linked insulin resistance, resulted in a decrease in amine stimulated glucose uptake by adipocytes (Mercier et al., 2003).

The remaining problem to be solved in terms of the physiological importance of these effects are how the levels of hydrogen peroxide formed by SSAO might be regulated. Apart from the plasma activator, referred to above, there is little known about factors that may increase the activity of the enzyme or its effective substrate affinity. The substrate concentrations that have been used in most in vitro studies are high (approximately mM) and there are no metabolic indications of whether substantial increases of substrate availability of substrate may accompany increased

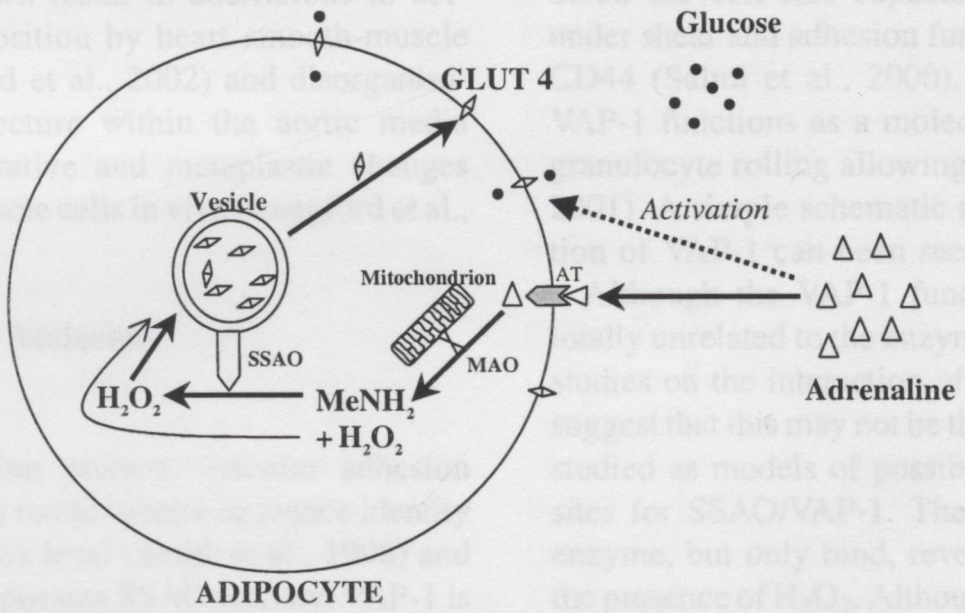


Fig. 3. Possible involvement SSAO in stimulating glucose transport by recruiting GLUT-4 transporters to the plasma membrane. Adrenaline uptake, through its transporter (AT), is followed by intracellular metabolism by mitochondrial monoamine oxidase to form methylamine ($MeNH_2$) which is then oxidized by SSAO. Both the MAO and SSAO reactions form hydrogen peroxide. The insulin involvement is omitted for clarity.

glucose demand. Since adrenaline stimulates glucose uptake by adipocytes and the breakdown of that amine by MAO releases methylamine, it is tempting to speculate that the increased metabolism of methylamine by SSAO might further enhance glucose transport capacity, as shown schematically in Fig. 3, but at the present state of our knowledge, that is no more than speculation.

Regulation of Cell Growth and Maturation

SSAO activity appears to play an important role in the development of some cell types. The levels in preadipocytes are essentially undetectable and increase to high levels during differentiation to mature adipocytes (Moldes et al., 1999; Raimondi et al., 1990). Although insulin is a stimulant for adipocyte differentiation, it does not induce SSAO expression (Enrique-Tarancon et al., 1998). Methylamine and other SSAO substrates were shown to induce maturation of adipocytes in a dose-dependent manner and since this effect was prevented by inhibition of SSAO or by treatment with an antioxidant it appears that the formation of H_2O_2 plays a key role (El Hadri et al., 2002; Fontana et al., 2001; Mercier et al., 2001). Intriguingly, although amines that are both substrates for MAO and SSAO, such as benzylamine, 2-phenethylamine and tyramine are effective in promoting adipocyte maturation, inhibition of MAO has rather little effect compared to that of SSAO inhibition (Mercier et al., 2001).

SSAO activity also plays an important role in extracellular matrix deposition and maintenance in vascular smooth muscle (Langford et al., 2002). Inhibition of SSAO have been shown result in aberrations in collagen and elastin deposition by heart smooth-muscle cells in vitro (Langford et al., 2002) and disorganisation of elastin architecture within the aortic media together with degenerative and metaplastic changes in vascular smooth muscle cells in vivo (Langford et al., 1999).

Actions of the SSAO Molecule

Cell Adhesion

Recently an adhesion protein, vascular adhesion protein-1 (VAP-1), was found to have sequence identity with SSAO at the cDNA level (Smith et al., 1998) and subsequently found to possess SSAO activity. VAP-1 is an endothelial glycoprotein that supports the adhesion of lymphocytes to endothelial cells and mediates lymphocyte re-circulation in a L-selectin dependent manner (see Jalkanen and Salmi, 2001 for review). VAP-1 has been shown to support sialic-acid dependent adhesion under shear stress and to mediate tethering to the tumour endothelium in human heptacellular carcinoma of T-cells (Yoong et al., 1998). Its cell-surface expression is induced under a number of inflammatory conditions, as shown in Table 1. There has been a flurry of publications on alterations in VAP-1 levels in disease states. Generally, these have shown the protein to behave in the same way as has been previously reported for SSAO. As discussed above, SSAO is elevated in diabetes and it has been suggested that the levels of plasma SSAO/VAP-1 are negatively regulated by insulin levels (Salmi et al., 2002). However, such a system does not explain the increased plasma SSAO in type 2, non-insulin dependent, diabetes. Insulin has also been shown to have no effects on the levels of adipocyte SSAO (Enrique-Tarancon et al., 1998). One notable difference is that VAP-1 has been reported to be upregulated by mediators associated with the inflammatory response such as interleukins 1 and 4, TNF-α, interferon-γ and lipopolysaccharide (Arvilommi et al., 1997), whereas, as discussed above, adipocyte SSAO has been shown to be downregulated by TNF-α.

Determination of the specificity for VAP-1 mediated adhesion showed that VAP-1 allows the initial binding of CD8-positive and natural killer cells (NKCs) to peripheral lymph node (PLN) high endothelial venules (HEVs) but this binding also required a peripheral lymph node addressin (PNAd) step (Salmi et al., 1998). VAP-1 shows selectivity, for example, discriminating between CD8- and CD16-positive effector cells. Transfection of VAP-1 cDNA into an endothelial cell line, which does not support cell adhesion, rendered the cell line capable of binding lymphocytes under shear and adhesion function was up regulated by CD44 (Salmi et al., 2000). Recent data suggest that VAP-1 functions as a molecular brake in vivo during granulocyte rolling allowing recruitment (Tohka et al., 2001). A simple schematic representation of the function of VAP-1 can been seen in Fig. 4.

Although the VAP-1 functions might appear to be totally unrelated to the enzymic functions of SSAO, our studies on the interaction of SSAO with amino sugars suggest that this may not be the case. Amino sugars were studied as models of possible cell-surface recognition sites for SSAO/VAP-1. They are not oxidised by the enzyme, but only bind, reversibly, to SSAO/VAP-1 in the presence of H_2O_2. Although externally added hydrogen peroxide does facilitate the amino sugar binding, that generated by the enzyme-catalysed oxidation of other substrates is considerably more effective in promoting this adhesion (O'Sullivan et al., 2003a, 2003b).

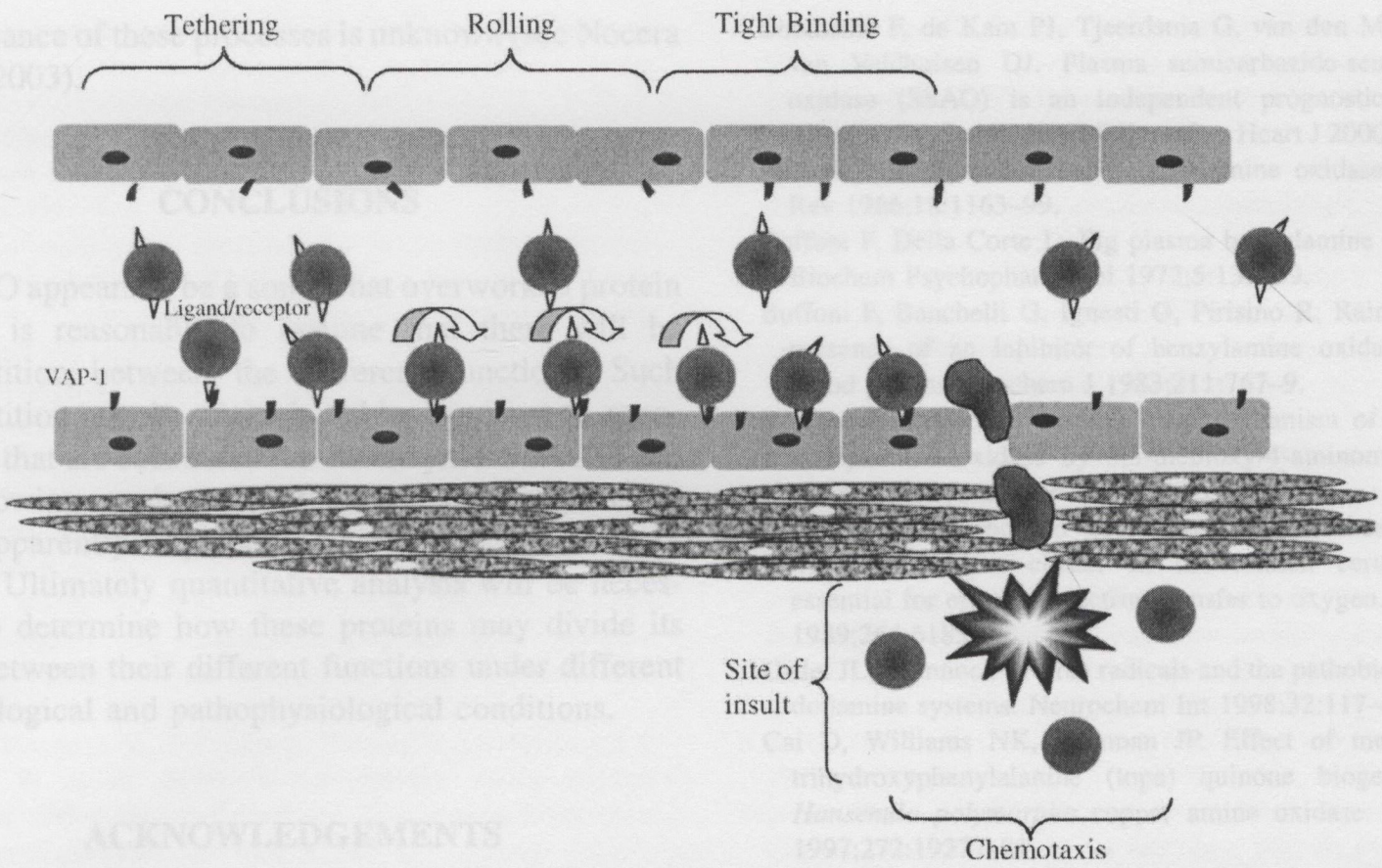


Fig. 4. The role of SSAO/VAP-1 in the extravasation of leukocytes to a site of injury or insult. The adhesion cascade is a multistep process. SSAO/VAP-1 is believed to mediate the leukocyte rolling and possibly to contribute to the extravasation of the cells into the tissue. Based on the model of Jalkanen and Salmi (2001).

The involvement of VAP-1 in mediating cell–cell adhesion raises questions about the possible function of the soluble, plasma, form of SSAO in this respect. It may act to modulate the actions of the membrane-bound protein, but the literature on this aspect is somewhat confusing (see, e.g. Jalkanen and Salmi, 2001). It is also unclear whether the membrane-bound SSAO in all other tissues has adhesion functions.

Drug Binding

Amiloride and some other imidazoline and guanidine compounds are inhibitors of sodium-transporting systems, which are useful as diuretic and hypertensive agents. Studies on the pharmacological behaviour of these drugs revealed the presence of tissue proteins, "amiloride binding proteins", that bound these drugs quite specifically. Semicarbazide sensitive amine oxidases (Novotny et al., 1994; Mu et al., 1994) and MAO (Limon-Boulez et al., 1996) may function in this way but the plasma and endothelial-surface location of SSAO may make it more important. Drug binding is reversible and inhibits the amine oxidase activity. The enzymes from different sources differ considerably in their binding abilities and SSAO from ox lung has, for example, been shown to have a low affinity (Lizcano et al., 1998). The possible significance of this binding for those taking such drugs remains unclear.

Lipid Trafficking

Studies with mature adipocytes have shown the surface SSAO to be located in caveolae. These caveolae are 50–100 nm invaginations of the plasma membrane that are formed by the expression of one or more isoforms of caveolin, the protein responsible for their distinct structure. It has been suggested that they are involved in lipid flux in and out of adipocytes. Their protein composition is limited and distinct. The major proteins present are the scavenger lipoprotein receptor, CD36 and SSAO. However, insulin receptors were found to be absent, as was the lipid-raft protein flotillin (Souto et al., 2003). These results suggest that adipocyte SSAO may be involved, in some as yet unidentified way, with lipid transport in the mature adipocyte.

Other Possible Functions

The semicarbazide-sensitive amine oxidase from bovine plasma has been reported to catalyse the oxidation of free amino groups in some small proteins including lysozyme and ribonuclease as well as polylysine (Wang et al., 1996). The same enzyme has been reported to be capable of modulating neuronal K^+ channel currents in a cell line. SSAO appears to enhance the channel currents by preventing ceruloplasmin-induced membrane depolarisation in a time dependent manner (Wu et al., 1996). The physiological

significance of these processes is unknown (see Nocera et al., 2003).

CONCLUSIONS

SSAO appears to be a somewhat overworked protein and it is reasonable to assume that there will be competition between the different functions. Such competition may be exacerbated by exposure to xenobiotics that are substrates for the enzyme. SSAO is one of a growing number of proteins that are recognised to have apparently disparate functions (see Tipton et al., 2003). Ultimately quantitative analysis will be necessary to determine how these proteins may divide its time between their different functions under different physiological and pathophysiological conditions.

ACKNOWLEDGEMENTS

We are grateful to the Health Research Board of Ireland, the Science Foundation of Ireland and to the EU COST D13 Programme for support.

REFERENCES

Alton G, Taher TH, Beever RJ, Palcic MM. Stereochemistry of benzylamine oxidation by copper amine oxidases. Arch Biochem Biophys 1995;353–61.

Andrés N, Lizcano JM, Rodríguez MJ, Romera M, Unzeta M, Mahy N. Tissue activity and cellular localization of human semicarbazide-sensitive amine oxidase. J Histochem Cytochem 2001;49:209–17.

Andrew R, Watson DG, Best SA, Midgley JM, Wenlong H, Petty KRH. The determination of hydroxydopamines and other trace amines in the urine of parkinsonian patients and normal controls. Neurochem Res 1993;18:1175–7.

Arvilommi AM, Salmi M, Jalkanen S. Organ-selective regulation of vascular adhesion protein-1 expression in man. Eur J Immunol 1997;27:1794–800.

Boomsma F, Derkx FH, van den Meiracker AH, Man in 't Veld AJ, Schalekamp MADH. Plasma semicarbazide-sensitive amine oxidase is elevated in diabetes mellitus and correlates with glycosylated haemogloblin. Clin Sci (Lond) 1995;88: 675–9.

Boomsma F, van Veldhuisen DJ, de Kam PJ, Man in 't Veld AJ, Mosterd A, Lie KI et al. Plasma semicarbazide-sensitive amine oxidase is elevated in patients with congestive heart failure. Cardiovasc Res 1997;33:387–91.

Boomsma F, van Dijk J, Bhaggoe UM, Bouhuizen AM, van den Meiracker AH. Variation in semicarbazide-sensitive amine oxidase activity in plasma and tissues of mammals. Comp Biochem Physiol C: Pharmacol Toxicol Endocrinol 2000a; 126:69–78.

Boomsma F, de Kam PJ, Tjeerdsma G, van den Meiracker AH, van Veldhuisen DJ. Plasma semicarbazide-sensitive amine oxidase (SSAO) is an independent prognostic marker for mortality in chronic heart failure. Eur Heart J 2000;21:1859–63.

Buffoni F. Histaminase and related amine oxidases. Pharmacol Rev 1966;18:1163–99.

Buffoni F, Della Corte L. Pig plasma benzylamine oxidase. Adv Biochem Psychopharmacol 1972;5:133–49.

Buffoni F, Banchelli G, Ignesti G, Pirisino R, Raimondi L. The presence of an inhibitor of benzylamine oxidase in human blood plasma. Biochem J 1983;211:767–9.

Buffoni F, Bertini V, Dini G. The mechanism of inhibition of benzylamine oxidase by 3,5-diethoxy-4-aminomethylpyridine (B24). J Enzyme Inhib 1998;13:253–66.

Calabrese L, Carbonaro M, Musci G. Presence of coupled trinuclear copper cluster in mammalian ceruloplasmin is essential for efficient electron transfer to oxygen. J Biol Chem 1989;264:6183–7.

Cadet JL, Brannock C. Free radicals and the pathobiology of brain dopamine systems. Neurochem Int 1998;32:117–31.

Cai D, Williams NK, Klinman JP. Effect of metal on 2,4,5-trihydroxyphenylalanine (topa) quinone biogenesis in the *Hansenula polymorpha* copper amine oxidase. J Biol Chem 1997;272:19277–81.

Callingham BA, McCarry WJ Barrand MA. Effects of some carbonyl reagents and short-acting MAO inhibitors on semicarbazide-sensitive (clorgyline-resistant) amine oxidase in the rat. In: Tipton KF, Dostert P, Strolin Benedetti M, editors. Monoamine oxidase and disease. Prospects for therapy with reversible inhibitors. London: Academic Press; 1984. p. 595–96.

Callingham BA, Crosbie AE, Rous BA. Some aspects of the pathophysiology of semicarbazide-sensitive amine oxidase enzymes. Prog Brain Res 1995;106:305–21.

Cronin CN, Zhang X, Thompson DA, McIntire WS. cDNA cloning of two splice variants of a human copper-containing monoamine oxidase pseudogene containing a dimeric Alu repeat sequence. Gene 1998;220:71–6.

Castillo V, Lizcano JM, Unzeta M. Presence of SSAO in human and bovine meninges and microvessels. Neurobiology 1999;7: 263–72.

Conklin DJ, Langford SD, Boor PJ. Contribution of serum and cellular semicarbazide-sensitive amine oxidase to amine metabolism and cardiovascular toxicity. Toxicol Sci 1998;46: 386–92.

Dalfó E, Hernandez M, Lizcano JM, Tipton KF, Unzeta M. Activation of human lung semicarbazide sensitive amine oxidase (SSAO) by a low molecular weight component present in human plasma. Biochim Biophys Acta 2003;1638:278–86.

Dutra F, Knudsen FS, Curi D, Bechara EJH. Aerobic oxidation of aminoacetone, a threonine catabolite: iron catalysis and coupled iron release from ferritin. Chem Res Toxicol 2001;14:1323–9.

Ekblom J, Gronvall JL, Garpenstrand H, Nillson S, Oreland L. Is semicarbazide-sensitive amine oxidase in blood plasma partly derived from the skeleton? Neurobiology 2000;8:129–35.

El Hadri K, Moldes M, Mercier N, Andreani M, Pairault J, Feve B. Semicarbazide-sensitive amine oxidase in vascular smooth muscle cells. Differentiation-dependent expression and role in glucose uptake. Arterioscler Thromb Vasc Biol 2002;22:89–94.

Elliott J, Callingham BA, Barrand MA. In vivo effects of (*E*)-2-(3′,4′-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) on

amine oxidase activities in the rat. Selective inhibition of semicarbazide-sensitive amine oxidase in vascular and brown adipose tissues. J Pharm Pharmacol 1989a;41:37–41.

Elliott J, Callingham BA, Sharman DF. Metabolism of amines in the isolated perfused mesenteric bed of the rat. Br J Pharmacol 1989b;98:507–14.

Elliott J, Callingham BA, Sharman DF. Amine oxidase enzymes of sheep blood vessels and blood plasma: a comparison of their properties. Comp Biochem Physiol C: Pharmacol Toxicol Endocrinol 1992;102:83–9.

Enrique-Tarancon G, Marti L, Morin N, Lizcano JM, Unzeta M, Sevilla L et al. Role of semicarbazide-sensitive amine oxidase on glucose transport and GLUT4 recruitment to the cell-surface in adipose cells. J Biol Chem 1998;273:8025–32.

Enrique-Tarancon G, Castan I, Morin N, Marti L, Abella A, Camps M et al. Substrates of semicarbazide-sensitive amine oxidase co-operate with vanadate to stimulate tyrosine phosphorylation of insulin–receptor–substrate proteins, phosphoinositide 3-kinase activity and GLUT4 translocation in adipose cells. Biochem J 2000;350:171–80.

Exner M, Hermann M, Hofbauer R, Kapiotis S, Quehenberger P, Speiser W et al. Semicarbazide-sensitive amine oxidase catalyzes endothelial cell-mediated low density lipoprotein oxidation. Cardiovasc Res 2001;50:583–8.

Ferrer I, Lizcano JM, Hernandez M, Unzeta M. Overexpression of semicarbazide sensitive amine oxidase in the cerebral blood vessels in patients with Alzheimer's disease and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Neurosci Lett 2002;321: 21–4.

Fitzgerald DH, Tipton KF. Inhibition of monoamine oxidase modulates the behaviour of semicarbazide-sensitive amine oxidase (SSAO). J Neural Transm 2002;109:251–65.

Fontana E, Boucher J, Marti L, Lizcano JM, Testar X, Zorzano A et al. Amine oxidase substrates mimic several of the insulin effects on adipocyte differentiation in 3T3 F442A cells (2001). Biochem J 2001;356:769–77.

Garpenstrand H, Ekblom J, Backlund LB, Oreland L, Rosenqvist U. Elevated plasma semicarbazide-sensitive amine oxidase (SSAO) activity in type 2 diabetes mellitus complicated by retinopathy. Diabet Med 1999a;16:514–21.

Garpenstrand H, Ekblom J, von Arbin M, Oreland L, Murray V. Plasma semicarbazide-sensitive amine oxidase in stroke. Eur Neurol 1999b;41:20–3.

Gewitz MH, Gillis CN. Uptake and metabolism of biogenic amines in the developing rabbit lung. J Appl Physiol 1981; 50:118–22.

Gronvall-Nordquist JL, Backlund LB, Garpenstrand H, Ekblom J, Landin B, Yu PH et al. Follow-up of plasma semicarbazide-sensitive amine oxidase activity and retinopathy in type 2 diabetes mellitus (2001). J Diabet Complic 2001;15:250–6.

Hasan F, Mc Crodden JM, Kennedy NP, Tipton KF. The involvement of intestinal monoamine oxidase in the transport and metabolism of tyramine. J Neural Transm 1998;26(Suppl): 1–9.

Heck HD, Casanova M, Starr TB. Formaldehyde toxicity—new understanding. Crit Rev Toxicol 1990;20:397–426.

Holt A, Callingham BA. Further studies on the ex vivo effects of procarbazine and monomethylhydrazine on rat semicarbazide-sensitive amine oxidase and monoamine oxidase activities. J Pharm Pharmacol 1995;47:837–45.

Holt AG, Alton G, Scaman CH, Loppnow GR, Szpacenko A, Svendsen I et al. Identification of the quinone cofactor in mammalian semicarbazide-sensitive amine oxidase. Biochemistry 1998;37:4946–57.

Houen G. Mammalian Cu-containing amine oxidases (CAOs): new methods of analysis, structural relationships, and possible functions. APMIS 1999;96:1–46.

Imamura Y, Kubota R, Wang Y, Asakawa S, Kudoh J, Mashima Y et al. Human retina-specific amine oxidases (RAO): cDNA cloning, tissue expression and chromosomal mapping. Genomics 1997;40:277–83.

Jalkanen S, Salmi M. Cell-surface monoamine oxidases: enzymes in search of a function. EMBO J 2001;20:3893–901.

Janes SM, Klinman JP. Isolation of 2,4,5-trihydroxyphenylalanine quinone (topa quinone) from copper amine oxidases. Meth Enzymol 1995;258:20–34.

Karádi I, Mészáros Z, Csányi A, Szombathy T, Hosszúfalusi L, Romics L et al. Serum semicarbazide-sensitive amine oxidase (SSAO) activity is an independent marker of carotid atherosclerosis. Clin Chim Acta 2002;323:139–46.

Klinman JP, Mu D. Quinoenzymes in biology. Ann Rev Biochem 1994;63:299–344.

Knowles PF, Yadav KDS. In: Lontie R, editor. Amine oxidases. Copper proteins and copper enzymes, vol. II. Flordia: CRC Press; 1984. p. 103–29.

Kurkijärvi R, Adams DH, Leino R, Mottonen T, Jalkanen S, Salmi M. Circulating form of human vascular adhesion protein-1 (VAP-1): increased serum levels in inflammatory liver diseases. J Immunol 1998;161:1549–57.

Kurkijärvi R, Jalkanen S, Isoniemi H, Salmi M. Vascular adhesion protein-1 (VAP-1) mediates lymphocyte–endothelial interactions in chronic kidney rejection. Eur J Immunol 2001;31: 2876–84.

Kinemuchi H, Jinbo M, Tabata A, Toyoshima Y, Arai Y, Tadano T et al. 2-Bromoethylamine, a suicide inhibitor of tissue-bound semicarbazide-sensitive amine oxidase. Jpn J Pharmacol 2000; 83:164–6.

Langford SD, Trent MB, Balakumaran A, Boor PJ. Developmental vasculotoxicity associated with inhibition of semicarbazide-sensitive amine oxidase. Toxicol Appl Pharmacol 1999;155:237–44.

Langford SD, Trent MB, Boor PJ. Semicarbazide-sensitive amine oxidase and extracellular matrix deposition by smooth-muscle cells. Cardiovasc Toxicol 2002;2:141–50.

Lewinsohn R. Mammalian monoamine-oxidizing enzymes, with special reference to benzylamine oxidase in human tissues. Braz J Med Biol Res 1984;17:223–56.

Lewinsohn R, Sandler M. Monoamine-oxidizing enzymes in human pregnancy. Clin Chim Acta 1982;120:301–12.

Limon-Boulez I, Tesson F, Gargalidis-Moudanos C, Parini A. I2-Imidazoline binding sites: relationship with different monoamine oxidase domains and identification of histidine residues mediating ligand binding regulation by H+1. J Pharmacol Exp Ther 1996;276:359–64.

Lizcano JM, Balsa D, Tipton KF, Unzeta M. Amine oxidase activities in bovine lung. J Neural Transm M 1990;32(Suppl): 341–4.

Lizcano JM, Balsa D, Tipton KF, Unzeta M. The oxidation of dopamine by the semicarbazide-sensitive amine oxidase (SSAO) from rat vas deferens. Biochem Pharmacol 1991a;41: 1107–10.

Lizcano JM, Escrich E, Ribalta T, Muntane J, Unzeta M. Amine oxidase activities in rat breast cancers induced experimentally with 7,12-dimethylbenz(alpha)anthracene. Biochem Pharmacol 1991b;42:263–9.

Lizcano JM, Fernandez de Ariba A, Tipton KF, Unzeta M. Inhibition of bovine lung semicarbazide-sensitive amine oxidase (SSAO) by some hydrazine derivatives. Biochem Pharmacol 1996;52:187–95.

Lizcano JM, Tipton KF, Unzeta M. Purification and characterization of membrane-bound semicarbazide-sensitive amine oxidase (SSAO) from bovine lung. Biochem J 1998; 331:69–78.

Lyles GA. Substrate-specificity of mammalian tissue-bound semicarbazide-sensitive amine oxidase. Progr Brain Res 1995; 106:293–303.

Lyles GA. Mammalian plasma and tissue-bound semicarbazide-sensitive amine oxidases: biochemical, pharmacological and toxicological aspects. Int J Biochem Cell Biol 1996;28:259–74.

Lyles GA, Chalmers J. The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. Biochem Pharmacol 1992;43:1409–14.

Lyles GA, Fitzpatrick CMS. An allylamine derivative (MDL 72145) with potent irreversible inhibitory actions on rat aorta semicarbazide-sensitive amine oxidase. J Pharm Pharmacol 1985;37:329–35.

Lyles GA, Marshall CMS, McDonald IA, Bey P, Palfreyman MG. Inhibition of rat aorta semicarbazide-sensitive amine oxidase by 2-phenyl-3-haloallylamines and related compounds. Biochem Pharnacol 1987;36:2847–53.

Marti L, Abella A, Carpene C, Palacin M, Testar X, Zorzano A. Combined treatment with benzylamine and low dosages of vanadate enhances glucose tolerance and reduces hyperglycemia in streptozotocin-induced diabetic rats. Diabetes 2001;50:2061–8.

Mathys KC, Ponnampalam SN, Padival S, Nagaraj RH. Semicarbazide-sensitive amine oxidase in aortic smooth muscle cells mediates synthesis of a methylglyoxal-AGE: implications for vascular complications in diabetes. Biochem Biophys Res Commun 2002;297:863–9.

McDonald IA, Lacoste JM, Bey P, Palfreyman MG, Zreika M. Enzyme-activated irreversible inhibition of monoamine oxidase: phenylallylamine structure–activity relationships. J Med Chem 1985;28:186–93.

McGuirl MA, Dooley DM. Copper-containing oxidases. Curr Opin Chem Biol 1999;3:138–44.

Mercier N, Moldes M, El Hadri K, Feve B. Semicarbazide-sensitive amine oxidase activation promotes adipose conversion of 3T3-L1 cells. Biochem J 2001;303:335–42.

Mercier N, Moldes M, Hadri KE, Feve B. Regulation of semicarbazide-sensitive amine oxidase expression by tumor necrosis factor-alpha in adipocytes: functional consequences on glucose transport. J Pharmacol Exp Ther 2003;304:1197–208.

Meszaros Z, Szombathy T, Raimondi L, Karadi I, Romics L, Magyar K. Elevated serum semicarbazide-sensitive amine oxidase activity in non-insulin-dependent diabetes mellitus: correlation with body mass index and serum triglyceride. Metabolism 1999;48:113–7.

Moldes M, Fève B, Pairault J. Molecular cloning of a major mRNA species in murine 3T3 adipocyte lineage: differentiation-dependent expression, regulation, and identification as semicarbazide-sensitive amine oxidase. J Biol Chem 1999;274: 9515–23.

Morgan DM. Polyamine oxidases. Biochem Soc Trans 1985;13:322–6.

Morin N, Lizcano JM, Fontana E, Marti L, Smih F, Rouet P et al. Semicarbazide-sensitive amine oxidase substrates stimulate glucose transport and inhibit lipolysis in human adipocytes. J Pharmacol Exp Ther 2001;297:563–72.

Mu D, Medzihradsezky KF, Adams GW, Mayer P, Hiness WM, Burlingames AL et al. Primary structures for a mammalian cellular and serum copper amine oxidase. J Biol Chem 1994; 269:9926–32.

Nilsson SE, Tryding N, Tufvesson G. Serum monoamine oxidase in diabetes mellitus and some other internal diseases. Acta Med Scand 1968;184:105–8.

Nocera S, Marcocci L, Pietrangeli P, Mondovi B. New perspectives on the role of amine oxidases in physiopathology. Amino Acids 2003;24:13–7.

Nordqvist A, Oreland L, Fowler CJ. Some properties of monoamine oxidase and a semicarbazide sensitive amine oxidase capable of the deamination of 5-hydroxytryptamine from porcine dental pulp. Biochem Pharmacol 1982;31:2739–44.

Nordquist JE, Gokturk C, Oreland L. Semicarbazide-sensitive amine oxidase gene expression in alloxan-induced diabetes in mice. Mol Med 2002;8:824–9.

Novotny WF, Chassande O, Baker M, Lazdunski M, Barbry P. Diamine oxidase is the amiloride-binding protein and is inhibited by amilorideanalogues. J Biol Chem 1994;269: 9921–5.

O'Sullivan M, Tipton KF, McDevitt WE. Immunolocalization of semicarbazide-sensitive amine oxidase in human dental pulp and its activity towards serotonin. Arch Oral Biol 2002;47: 399–406.

O'Sullivan J, O'Sullivan M, Tipton KF, Unzeta M, Hernandez M, del M et al. The inhibition of semicarbazide-sensitive amine oxidase by aminohexoses. Biochim Biophys Acta 2003a;1647: 367–71.

O'Sullivan M, MacDougall MB, Unzeta M, Lizcano JM, Tipton KF. Semicarbazide-sensitive amine oxidases in pig dental pulp. Biochim Biophys Acta 2003b;1647:333–6.

Palfreyman MG, McDonald IA, Bey P, Danzin C, Zreika M, Cremer G. Haloallylamine inhibitors of MAO and SSAO and their therapeutic potential. J Neural Transm 1994;41(Suppl): 407–14.

Precious E, Gunn CE, Lyles GA. Deamination of methylamine by semicarbazide-sensitive amine oxidase in human umbilical artery and rat aorta. Biochem Pharmacol 1988;37:707–13.

Raimondi L, Pirisino R, Banchelli G, Ignesti G, Conforti L, Buffoni F. Cultured preadipocytes produce a semicarbazide-sensitive amine oxidase (SSAO) activity. J Neural Transm 1990;331–36.

Ramos KSL, Grossman SL, Cox LR. Allylamine-induced vascular toxicity in vitro: prevention by semicarbazide-sensitive amine oxidase inhibitors. Toxicol Appl Pharmacol 1988;95:61–71.

Rydén L. Ceruloplasmin. In: Lontie, L, editor. Copper proteins and copper enzymes copper proteins and copper enzymes. Boca Raton FL, USA: CRC Press; 1984. p. 37–100.

Salmi M, Hellman J, Jalkanen S. The role of two distinct endothelial molecules, vascular adhesion protein-1 and peripheral lymph node addressin, in the binding of lymphocyte subsets to human lymph nodes. J Immunol 1998;160:5629–36.

Salmi M, Tohka S, Jalkanen S. Human vascular adhesion protein-1 (VAP-1) plays a critical role in lymphocyte-endothelial cell adhesion cascade under shear. Circ Res 2000;86:1245–51.

Salmi M, Stolen C, Jousilahti P, Yegutkin GG, Tapanainen P, Janatuinen T et al. Insulin-regulated increase of soluble vascular adhesion protein-1 in diabetes. Am J Pathol 2002;161:2255–62.

Salminen TA, Smith DJ, Jalkenen S, Johnson MS. Structural model of the catalytic domain of an enzyme with cell adhesion activity: human vascular adhesion protein-1 (HVAP-1). Protein Eng 1998;11:1195–204.

Sikkema JM, Franx A, Fijnheer R, Nikkels PG, Bruinse HW, Boomsma F. Semicarbazide-sensitive amine oxidase in pre-eclampsia: no relation with markers of endothelial cell activation. Clin Chim Acta 2002;324:31–8.

Smith DJ, Salmi M, Bono P, Hellman J, Leu T, Jalkanen S. Cloning of vascular adhesion protein 1 reveals a novel multifunctional adhesion molecule. J Exp Med 1998;188:17–27.

Smith-Mungo LI, Kagan HM. Lysyl oxidase: properties, regulation and multiple functions in biology. Matrix Biol 1988;16:387–98.

Souto RP, Vallega G, Wharton J, Vinten J, Tranum-Jensen J, Pilch PF. Immunopurification and characterization of rat adipocyte caveolae suggest their dissociation from insulin signalling. J Biol Chem 2003;278:18321–9.

Thornalley PJ. Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. Gen Pharmacol 1996;27:565–73.

Tipton KF, Strolin Benedetti M. Amine oxidases and the metabolism of xenobiotics. In: Ioannides C, editor. Enzyme systems that metabolise drugs and other xenobiotics. Chichester: Wiley; 2001. p. 95–146.

Tipton KF, Davey G, Motherway M. Monoamine oxidase assays. In: Taylor GP, Sullivan JP, editors. Current protocols in pharmacology. New York: Wiley; 2000. p. 3.6.1–42.

Tipton KF, O'Sullivan MI, Davey GP, O'Sullivan J. It can be a complicated life being an enzyme. Biochem Soc Trans 2003;31:711–5.

Tohka S, Laukkanen M-L, Jalkanen S, Salmi M. Vascular adhesion protein 1 (VAP-1) functions as a molecular brake during granulocyte rolling and mediates recruitment in vivo. FASEB J 2001;15:373–82.

Wang XT, Pietrangeli P, Mateescu M, Mondovì B. Extended substrate specificity of serum amine oxidase: possible involvement in protein posttranslational modification. Biochem Biophys Res Commun 1996;223:91–7.

Wang SX, Nakamura N, Mure M, Klinman JP, Sanders-Loeht J. Characterization of the native lysine tyrosylquinone cofactor of lysyl oxidase by Raman spectroscopy. J Biol Chem 1997;272: 28841–4.

Wilmot CM, Murray JM, Alton G, Parsons MR, Convery MA, Blakeley V et al. Catalytic mechanism of the quinoenzyme amine oxidase from *Escherichia coli*: exploring the reductive half-reaction. Biochemistry 1997;36:1608–20.

Wu L, Mateescu MA, Wang XT, Mondovì B, Wang R. Modulation of K^+ channel currents by serum aminoxidase in neurons. Biochem Biophys Res Commun 1996;220:47–52.

Yang FM, Friedrichs WE, Cupples RL, Bonifacio MJ, Sanford JA, Horton WA, Bowman BH. Human ceruloplasmin. Tissue-specific expression of transcripts produced by alternative splicing. J Biol Chem 1990;265:10780–5.

Yoong KF, McNab G, Hübscher SG, Adams DH. Vascular adhesion protein-1 and I-CAM-1 support the adhesion of tumor-infiltrating lymphocytes to tumor endothelium in human hepatocellular carcinoma. J Immunol 1998;160:3978–88.

Young SN, Davis BA, Gauthier S. Precursors and metabolites of phenylethylamine, *m*- and *p*-tyramine and tryptamine in human lumbar and cisternal cerebrospinal fluid. J Neurol Neurosurg Psychiatry 1982;45:633–9.

Yu PH. Deamination of methylamine and angiopathy; toxicity of formaldehyde, oxidative stress and relevance to protein glycoxidation in diabetes. J Neural Transm 1998;52(Suppl): 201–16.

Yu PH, Davis BA. Stereospecific deamination of benzylamine catalyzed by different amine oxidases. Int J Biochem 1988; 20:1197–201.

Yu PH, Deng Y. Potential cytotoxic effect of chronic administration of creatine, a nutrition supplement to augment athletic performance. Med Hypotheses 2000;54:726–8.

Yu PH, Davis BA, Deng Y. 2-Bromoethylamine as a potent selective suicide inhibitor for semicarbazide-sensitive amine oxidase. Biochem Pharmacol 2001;61:741–8.

Zhang X, McIntire WS. Cloning and sequencing of a human copper-containing, topa quinone-containing monoamine oxidase from human placenta. Gene 1996;179:279–86.

Zreika M, McDonald IA, Bey P, Palfreyman MG. MDL 72145, an enzyme-activated irreversible inhibitor with selectivity for monoamine oxidase type B. J Neurochem 1984;43:448–54.