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THE EFFECT OF ACUTE TRAUMATIC BRAIN INJURY ON CARDIOVASCULAR HOMEOSTASIS

Dr Christine Geraldine McMahon, MB, BAO, BCh, BScAnat, FRCSEd, FRCPI, FCEM.

Submitted for the award of PhD of the University of Dublin, Trinity College.

Supervisors

Rose Anne Kenny, MD, FRCP, FRCPI.
Professor of Geriatric Medicine,
Institute of Neuroscience, Trinity College, Dublin 2, Ireland

Emrys Kirkman, PhD.
Principal Scientist,
Biophysics & Trauma (Surgical Science), Biomedical Sciences,Dstl Porton Down, Salisbury
SP4 OJQ, UK
Declaration

I certify that this thesis which I now submit for examination for the award of PhD, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for post graduate study of the University of Dublin, Trinity College, and has not been submitted in whole or in part for an award in any other institute or University.

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Signature .................................................. Date..............................
Dedications

This work is dedicated to my husband Ronan, daughter Síofra, and sons Ronan and David.
Acknowledgements

I would like to sincerely thank both of my supervisors, Professor Rose Anne Kenny and Dr Emrys Kirkman for their mentorship, expert advice and support. They have taught me more than research skills alone.

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Finally, I would like to thank the Medical Research Council of United Kingdom, who provided the funding support for the laboratory studies undertaken for this research.
Summary

Acute traumatic brain injury (TBI) remains the most common cause of death from multiple trauma. The cardiovascular homeostatic responses to haemorrhage and injury are co-coordinated in the central nervous system. Coincidental brain injury, which is present in at least 21% of trauma patients, could impair these responses and thereby adversely affect outcome. The effects of acute TBI on cardiovascular homeostatic mechanisms are unclear.

The first objective of this research was to test the hypothesis that acute TBI of moderate severity (Abbreviated Injury Score=3) impairs survival from multiple trauma. Analysis of the UK trauma audit and research network database was undertaken in a study population of multiple trauma patients who had sustained blunt trauma with an injury severity score (ISS) ranging between 16 and 50. These parameters were chosen to reflect an injury severity where acute TBI could affect mortality. The probability of death was modeled using logistic regression adjusted for age and injury severity. Moderate TBI in isolation was associated with a low mortality rate (< 4.7%). Adjusting for ISS, mortality from multiple trauma was doubled in the presence of moderate TBI (odds ratio 2.08, 95% CI, 1.57-2.77). This observation led to the hypothesis that acute TBI could modify cardiovascular homeostasis, and thereby contribute to the additional mortality observed when TBI and extracranial injuries are combined.

The effect of mild and moderate TBI on the neurally-mediated bi-phasic haemodynamic response to haemorrhage was examined using a preclinical model of closed head injury and simple haemorrhage in the rat. The lateral fluid percussion injury model of brain trauma was combined with a non-resuscitative, controlled haemorrhage model of 40% total blood volume. TBI resulted in a significant attenuation of the bi-phasic haemodynamic response to simple haemorrhage. This effect was graded according to the severity of induced brain injury. Moderate TBI was associated with a marked attenuation of the bi-phasic response, with heart rate and blood pressure being maintained higher for longer. Despite this apparent improved tolerance for haemorrhage, a 50% early mortality was observed following moderate TBI. Subgroup analysis of the moderate TBI group revealed that the bi-phasic haemodynamic response...
was abolished in non-survivors. These findings reflect a significant disturbance of normal cardiovascular homeostasis following acute TBI.

The performance of Shock Index in the presence of combined TBI and haemorrhage was assessed. Shock Index failed to reflect the trend of increasing blood volume loss in the presence of TBI. Shock Index appears to be an unreliable indicator of early shock in the presence of acute TBI.

The arterial baroreflex is the one of the principal reflexes involved in the moment to moment control of blood pressure. The effects of acute TBI on the arterial baroreflex were studied using the rapid phenylephrine pressor test. Acute TBI was associated with a significant increase in baroreflex sensitivity within 10 minutes of induced brain injury. This increase in baroreflex sensitivity was closely related to the severity of induced TBI and was observed in both mild and moderate severities of acute TBI. Future research will be required to determine the implications of this alteration in baroreflex sensitivity on haemodynamic stability in the brain injured patients.

In summary, acute TBI is associated with a significant adverse effect on survival following multi-system trauma. Laboratory investigations revealed a significant disturbance of the basic cardiovascular homeostatic mechanisms complicating even mild TBI. Further clinical and laboratory research introducing more complex preclinical and clinical investigations are needed to advance our understanding of cardiovascular pathophysiology in brain injured patients and thereby improve outcomes in this group.
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<th>Definition</th>
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<tbody>
<tr>
<td>AIS</td>
<td>Abbreviated Injury Scale</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>atm</td>
<td>Atmosphere</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CVLM</td>
<td>Caudal ventrolateral medulla</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CT</td>
<td>Computerised tomography</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral Perfusion Pressure</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>DAI</td>
<td>Diffuse axonal injury</td>
</tr>
<tr>
<td>DVMN</td>
<td>Dorsal vagal motor nucleus</td>
</tr>
<tr>
<td>EDCFs</td>
<td>Endothelium-derived contracting factors</td>
</tr>
<tr>
<td>EBIC</td>
<td>European Brain Injury Consortium</td>
</tr>
<tr>
<td>GCS</td>
<td>Glasgow Coma Score</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>ISS</td>
<td>Injury Severity Score</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IML</td>
<td>Intermediolateral</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
</tr>
<tr>
<td>LFP</td>
<td>Lateral fluid percussion</td>
</tr>
<tr>
<td>LVSW</td>
<td>Left ventricular stroke work</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>LBNP</td>
<td>Lower body negative pressure</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>msec</td>
<td>Milliseconds</td>
</tr>
<tr>
<td>NO</td>
<td>Nitrous oxide</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NA</td>
<td>Nucleus Ambiguus</td>
</tr>
<tr>
<td>NTS</td>
<td>Nucleus Tractus Solitarius</td>
</tr>
<tr>
<td>PGL</td>
<td>Paragigantocellularis Lateralis</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular Nucleus</td>
</tr>
<tr>
<td>PAG</td>
<td>Periaqueductal grey</td>
</tr>
<tr>
<td>RTS</td>
<td>Revised Trauma Score</td>
</tr>
<tr>
<td>RVLM</td>
<td>Rostral Ventrolateral Medulla</td>
</tr>
<tr>
<td>RVMM</td>
<td>Rostral Ventromedial Medulla</td>
</tr>
<tr>
<td>SI</td>
<td>Shock index</td>
</tr>
<tr>
<td>SE</td>
<td>Standard errors</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard errors of mean</td>
</tr>
<tr>
<td>SOP</td>
<td>Supraoptic</td>
</tr>
<tr>
<td>SPN</td>
<td>Sympathetic preganglionic neurones</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>TPR</td>
<td>Total peripheral resistance</td>
</tr>
<tr>
<td>TBI</td>
<td>Traumatic brain injury</td>
</tr>
<tr>
<td>TRISS</td>
<td>Trauma Score, Injury Severity Score &amp; Age combined</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TARN</td>
<td>Trauma audit research network</td>
</tr>
<tr>
<td>VSM</td>
<td>Vascular smooth muscle cells</td>
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CHAPTER 1

INTRODUCTION

1.1 EPIDEMIOLOGY

Trauma remains the leading cause of death and disability in the first four decades of life in western societies. It is the third most common cause of death in all age groups (1988), (Murray 1997). Over 300,000 people are severely injured and over 10,000 people die as a result of their injuries every day (Murray 1997), (Lopez, Mathers et al. 2006). Traumatic brain injury (TBI) is the leading cause of death following injury, accounting for over 50% of all trauma deaths (Kraus and McArthur 1996).

The incidence estimates of TBI are in the range 200-300 per 100,000 population per year based on hospital admission figures (Murray 1997). In terms of the severity of brain injury sustained approximately 80% are mild, 10% are moderate and 10% are severe (Jennett 1996). The incidence rates for males are twice those of females. Age data typically show two peaks; one in the 15-24 year age group and the other in the over 70 year age group. TBI has significant implications for public health, contributing to acute health care and long-term disability costs in a predominantly younger age group (Murray, Teasdale et al. 1999), (Tagliaferri, Compagnone et al. 2006). The European Brain Injury Consortium (EBIC) survey of over 1,000 patients with moderate to severe TBI showed that 31% were dead within 6 months, 30% made a good recovery, 20% were moderately disabled, 16% were severely disabled and 3% were in a vegetative condition (Murray, Teasdale et al. 1999).
Road traffic accidents, falls and assaults are the main causes of TBI. In the EBIC survey 52% of injuries were road traffic accident related (Murray, Teasdale et al. 1999). There is a clear relationship between age and the mechanism of injury. Younger patients are more frequently injured in road traffic accidents (83%). The incidence of falls and pedestrian accidents increases with age accounting for 82.9% of injuries in patients over the age of 65 years. Advancing age is also an independent risk factor for poor outcome following acute TBI (Teasdale, Skene et al. 1979), (Susman, DiRusso et al. 2002), (Kuhne, Ruchholtz et al. 2005), (Franko, Kish et al. 2006), (Thompson, McCormick et al. 2006).

Epidemiological studies indicate that improved systems of trauma care have resulted in a 40% reduction in mortality from major trauma when there is no associated brain injury (Shackford, Mackersie et al. 1987), (Davis, Hoyt et al. 1991), (Lecky, Woodford et al. 2000), (Hartl, Gerber et al. 2006), (Hartl, Gerber et al. 2006). Significant improvements in survival from isolated brain injury have also been observed over the past 25 years, with a reduction in mortality from severe TBI from 50% to 25% (Marshall 2000), (Zink 2001). This improvement in survival is much less evident when acute TBI is a component of multiple trauma (Gennarelli, Champion et al. 1989), (Patel, Bouamra et al. 2005). Therefore the interaction between acute TBI and extracranial injury has a deleterious effect on outcome.

Extracranial injuries commonly complicate acute TBI and approximately 50% of patients with severe TBI sustain potentially life-threatening extracranial injuries (Luerssen, Klauber et al. 1988), (Sarrafzadeh, Peltonen et al. 2001). The combination of
TBI with extracranial injuries, is associated with a high incidence of secondary insults (Miller 1982), (Stocchetti 1996), (Sarrafzadeh, Peltonen et al. 2001).

Secondary ischaemic insults to injured neuronal tissue are considered key factors contributing to the additional mortality observed when acute TBI co-exists with extracranial injury (Schmoker, Zhuang et al. 1992), (Chesnut, Marshall et al. 1993), (Signorini, Andrews et al. 1999), (Jeremitsky, Omert et al. 2003). Ischaemic brain injury is present in up to 90% of patients who die as a result of their injuries (Graham and Adams 1971), (Kotapka, Gennarelli et al. 1991), (Wald, Shackford et al. 1993).

Furthermore, acute TBI may be associated with concomitant injury to critical sites of the central nervous system responsible for homeostatic responses to extracranial haemorrhage and soft tissue injury. Despite the advances in emergency and critical care, patients with acute TBI continue to experience frequent hypotensive episodes following their injury (Jones, Andrews et al. 1994), (Jeremitsky, Omert et al. 2003). It is possible that injury to critical sites of the central nervous system responsible for cardiovascular control may in part account for this tendency to hypotensive episodes. However the effect of acute TBI on cardiovascular homeostasis remains poorly understood. Hypotensive insults are an important negative predictor of outcome following TBI (Schmoker 1992), (Stocchetti 1996), (Chesnut 1997), (Jeremitsky, Omert et al. 2003). Mortality increases by 50% when even a brief episode of hypotension occurs (MAP of <90mmHg) (Jones, Andrews et al. 1994), (Chesnut 1997).

It is hypothesised that acute TBI may be associated with modification of the normal cardiovascular homeostatic mechanisms and may therefore contribute to the observed
additional mortality seen when acute TBI co-exists with extracranial injury. This may also contribute to the frequent episodes of hypotension which occur in TBI patients despite targeted therapy aimed at minimising secondary insults of this type (Jones, Andrews et al. 1994), (Jeremitsky, Omert et al. 2003).

In the remainder of this chapter an overview of the relevant aspects of the neural mechanisms of cardiovascular homeostasis and the pathophysiology of primary and secondary TBI are discussed. Finally the core aims of this thesis are presented.
1.2 THE NEURAL MECHANISMS INVOLVED IN CARDIOVASCULAR HOMEOSTATIC RESPONSES TO HAEMORRHAGE.

The central nervous system sets the balance of sympathetic and parasympathetic activity which determines cardiac output and peripheral vascular resistance and hence blood pressure. Peripheral receptors monitor the level of arterial pressure and contribute reflexly to the regulation of arterial pressure. The interplay of a ‘central command’ and peripheral sensors with reflex inputs is essential for the moment to moment adjustment of blood pressure.

Victims of multi-system trauma often sustain two different types of extracranial insults: simple haemorrhage and soft tissue (musculoskeletal) injury. The cardiovascular reflex responses to simple haemorrhage differ from the responses to soft tissue injury (Little and Stoner 1983), (Little, Randall et al. 1984), (Anderson, Little et al. 1990), (Evans, Ventura et al. 2001). When both of these injuries co-exist they interact to produce a third pattern of cardiovascular reflex responses (Little, Marshall et al. 1989).

1.2.1 CARDIOVASCULAR REFLEX RESPONSES TO SIMPLE HAEMORRHAGE

Simple haemorrhage, defined as loss of circulating blood volume in the absence of soft tissue injury, triggers a number of reflex responses which results in a bi-phasic heart rate (HR) and blood pressure response (Barcroft, Edholm et al. 1944), (Warren 1945), (Secher and Bie 1985), (Evans, Ventura et al. 2001), (Figure 1). Three principal reflexes are considered to be involved in the cardiovascular response to haemorrhage; the arterial baroreflex, the cardiac C fibre reflex and the chemoreceptor reflex.
Response to venesection in humans: illustrating the 2 phases of the response to acute hypovolaemia. During phase 1, arterial pressure is maintained in the presence of progressively reduced cardiac output (CO; ▲), by increased total peripheral resistance (TPR; ● ). The onset of phase 2 occurs abruptly once CO has reached a critical level and is characterised by a dramatic reduction in TPR and arterial pressure and invariably the subject faints. Heart rate (HR; ◇ ); systolic blood pressure (SBP; ● ); right auricular pressure (RAP; □ ).
1.2.1.1 Arterial Baroreceptor Reflex

The arterial baroreflex is the primary cardiovascular reflex responsible for the maintenance of arterial blood pressure in the presence of acute central hypovolaemia resulting from haemorrhage or soft tissue injury (Schadt and Ludbrook 1991), (Potts, Ludbrook et al. 2000), (Evans, Ventura et al. 2001). Modification of arterial baroreflex function following acute TBI could have significant implications for cardiovascular compensation for blood volume loss following multi-system trauma. The arterial baroreceptors are slow-adapting ‘mechanoreceptors’ that respond to the degree of stretch of the arterial wall produced by intraluminal pressure. The main baroreceptors involved in monitoring the arterial circulation are strategically located in the medio-adventitial layer of the walls of the aortic arch and carotid sinus area (Angell-James and Daly 1970).

Baroreceptors respond both to the degree of stretch of the arterial wall and to the rate of change of pulse pressure and mean arterial pressure (MAP). Baroreceptors are tonically active at a MAP above 70mmHg, and are inactive at a MAP below 50mmHg. As pulse pressure diminishes following haemorrhage the activity of the baroreceptors reduces even before a fall in MAP occurs. Two types of afferent nerve fibres are associated with the arterial baroreflex; the majority are A-type (myelinated) nerve fibres and there are a smaller number of C-type (non-myelinated) fibres. A-fibres are considered to provide a dynamic reflex modulation whilst C-fibres are thought to provide a more steady-state response (Fan 1999). Sensory fibres from the aortic arch baroreceptors travel in the vagus nerve and from the carotid sinus in the sinus nerve (Van Hering’s nerve), a branch of the glossopharyngeal nerve. They relay in the nodose ganglia and
the petrosal ganglia respectively, before terminating in the nucleus of the tractus solitarius (NTS) which is located in the dorsomedial medulla (Kirchheim 1976). When arterial pressure is increased, the sensory nerve endings of the baroreceptors are stretched and afferent discharge is increased.

Conversely when the degree of stretch is diminished the afferent activity of baroreflex is reduced or ‘unloaded’. The efferent limb of the baroreflex is carried in the vagus and sympathetic nerves to the heart, and in the sympathetic vasoconstrictor nerves to the blood vessels (Kirchheim 1976). Stimulation of the baroreceptors results in a reflex bradycardia and fall in peripheral vascular resistance and hypotension, whilst unloading the arterial baroreceptors has the opposite effect (Smyth, Sleight et al. 1969), (Pickering, Gribbin et al. 1972). This forms the basis of the negative feedback mechanism of the arterial baroreflex which is considered to be one of the most important mechanisms involved in the ‘moment to moment’ control of arterial blood pressure (Cowley, Liard et al. 1973).

Whilst the baroreflex is one of the principal mechanisms for controlling and maintaining blood pressure, it is not the only mechanism. The overall control of autonomic system is more complex. When the need arises other mechanisms such as the ‘defence reaction’ and the response to ‘injury’ can assume control and effectively override the ‘normal’ responses, resulting in a centrally mediated attenuation of the baroreflex (Hilton 1982). There is, therefore, a hierarchical system of control and adaptability of cardiovascular responses within the central nervous system.
Figure 2. Anatomical location of the arterial baroreceptors and chemoreceptors.

Sensory fibres from the aortic arch baroreceptors travel in the vagus nerve. Carotid sinus baroreceptors are located at the internal carotid artery after the bifurcation of the common carotid artery. Sensory fibres from the carotid sinus travel in the sinus nerve (Van Hering’s nerve), a branch of the glossopharyngeal nerve. Chemoreceptors are located in the carotid and aortic bodies in close proximity to the carotid sinus and the aortic arch. Afferents from the carotid sinus travel in the carotid sinus nerve and the glossopharyngeal nerve, afferents from the aortic bodies travel in the vagus nerves.
1.2.1.2 The Arterial Chemoreceptor Reflex

As haemorrhage progresses and MAP falls below 50mmHg arterial baroreceptors become insensitive. At this lower range of blood pressure carotid body chemoreceptors are activated. Chemoreceptors are located in the carotid and aortic bodies in close proximity to the carotid sinus and the aortic arch. They respond to changes in oxygen tension in arterial blood. A fall in oxygen tension results in an increase in chemoreceptor activity. Their sensitivity is increased by increases in carbon dioxide tension and a reduction in arterial pH. Sensory fibres from the carotid body travel in the carotid sinus nerve, a branch of the glossopharyngeal nerve to the brainstem, from where the vagal and sympathetic efferent activity is modulated.

The main cardiovascular effects of increased chemoreceptor activity are a vagally mediated bradycardia and an increase in sympathetic tone in skeletal muscle. The arterial chemoreceptor afferents terminate within the NTS. From the NTS there is a secondary excitatory projection activating both the vagal cardiac preganglionic motor neurones and the inspiratory neurones within the nucleus ambiguus (NA), resulting in an increased respiratory rate (Biscoe, Bradley et al. 1970). The inspiratory neurones send cholinergic inhibitory collaterals onto the vagal cardiac pre-ganglionic motor neurones which act to attenuate the chemoreceptor-induced increased vagal efferent activity to the heart. Respiratory stimulation also results in activation of the lung stretch afferent fibres, which has the effect of inhibiting the vagal activity to the heart and the sympathetic vasoconstrictor tone in skeletal muscle (Spyer 1984). Severe haemorrhage, therefore causes activation of the chemoreceptors resulting in an increase respiratory
rate, tachycardia and a reduction in vasoconstriction in skeletal muscle (Daly and Kirkman 1988).

1.2.1.3 Phase 1 – sympathoexcitatory phase of the response to haemorrhage

With the onset of progressive simple haemorrhage, pulse pressure diminishes resulting in ‘unloading’ of baroreceptor afferent activity. Reduced afferent baroreflex activity results in a reflex withdrawal of vagally-mediated cardiac tone causing an increase in HR and a sympathetically-mediated increase in resistance in peripheral vessels such that blood pressure is maintained close to pre-haemorrhage levels. This response maintains MAP and oxygen delivery to organs with higher metabolic requirements and low anaerobic reserve such as the brain, heart and kidneys at the expense of other tissues such as the skin and skeletal muscle (Mackway-Jones, Foex et al. 1999). It is the arterial baroreflex response to reduced arterial pressure that almost exclusively accounts for Phase 1 of the response to haemorrhage (Schadt and Ludbrook 1991), (Ludbrook and Ventura 1996), (Evans, Ventura et al. 2001). This reflex response maintains MAP close to pre-haemorrhage levels following blood loss of up to 10-15% in an otherwise healthy individual (Barcroft, Edholm et al. 1944), (Secher and Bie 1985).

The activity of the baroreflex is augmented as haemorrhage continues by a concomitant increase in the sensitivity. This is the result of an increased secretion of vasopressin and renin, which occurs after haemorrhage (Scott and Kirkman 1983), (Cowley, Merrill et al. 1984). The cell bodies of the sympathetic preganglionic neurones, which cause vasoconstriction, are located in the intermediolateral (IML) column of the spinal cord. One of the main pathways of excitatory drive to the sympathetic pre-ganglionic
neurones originates in the nucleus paragigantocellularis lateralis (PGL) in the rostral ventrolateral medulla (RVLM). These sympato-excitatory cells within the RVLM are subject to inhibition originating from baroreceptor afferent activity. The reduction in baroreceptor afferent activity which occurs during haemorrhage results in a disinhibition of the RVLM sympatoexcitatory neurones which therefore results in an increase in sympathetic activity (Pilowsky 2002).

At a micro-vascular level the balance of pressures across the capillary beds changes with haemorrhage. Fluid shifts result in some movement of fluid from the extravascular space to the intravascular compartment to further minimise reductions in blood pressure resulting from blood loss. As blood loss progresses beyond 20-30% of total blood volume, there is a failure of sympathetic vasoconstrictor tone and an increase in cardiac vagal tone resulting in a dramatic fall in blood pressure. This is due to the activation of a second reflex, the ‘depressor’ reflex (Phase 2).

1.2.1.4 Phase 2 – Sympathoinhibitory phase.

The neural mechanisms that trigger the onset of the second phase of the response to acute central hypovolaemia are not fully understood. There is evidence that the sympathoinhibitory phase or the ‘depressor response’ associated with severe haemorrhage is reflex in nature and that the efferent limb of the reflex involves both increased vagal activity to the heart and reduced sympathetic tone to the vascular beds. The decrease in blood pressure appears to be independent of the decrease in HR as it is unaffected by the administration of atropine (Barcroft, Edholm et al. 1944), (Murray and Shropshire 1970). There is little information, however, about the neuroanatomy of
this presumptive ‘cardiac reflex’. There are some studies that reveal aspects of the
neuroanatomy of this reflex. Hypotensive haemorrhage increases activity in a number
of midbrain (supraoptic (SOP), paraventricular (PVN), periaqueductal grey (PAG)) and
brainstem nuclei (NTS, RVLM), some of which project to the spinal cord. The
possibility that these suprapontine centres play a role in the depressor phase of the
cardiovascular response to severe haemorrhage is supported by the observation that
phase 2 is abolished by high mesencephalic decerebration in rats (Evans, Ludbrook et

It has been postulated that the mechano-sensitive receptor endings of the cardiac vagal
C-fibre afferents are stimulated by deformations of the ventricular wall as the heart
contracts around an incompletely filled ventricle (Thorén 1979). In support of this
theory sectioning of the cervical vagii reverses the bradycardia in experimental animals
(Evans 1994). The bradycardia and hypotension at this phase are attenuated in animals
deficient in afferent C-fibres (Little, Marshall et al. 1989). However phase 2 still occurs
after vagotomy (Morita and Vatner 1985) or cardiac denervation (Shen, Knight et al.
1990) in conscious dogs and in humans, and a ‘depressor-like’ response occurs in heart
transplant recipients (Fitzpatrick, Banner et al. 1993), (Morgan-Hughes, Kenny et al.
1994). Regardless of the aetiology of the afferent pathway, the efferent pathway of the
‘depressor reflex’ involves both increased vagal activity to the heart and reduced
sympathetic activity to vascular beds which occurs when a critical level of blood loss
takes place (Oberg and Thorén 1973).
1.2.2 Central Organisation of Cardiovascular Regulatory Nuclei

Research over the past 50 years has greatly enhanced our knowledge of the central organisation of cardiovascular regulation. An overview of the key aspects of the central organisation of cardiovascular reflex mechanisms is outlined in the next section.

1.2.2.1 Afferent Medullary Nuclei

Afferent inputs from the arterial baroreceptors and a range of other peripheral receptors influence the activity of cardiovascular autonomic nerves. These include the cardiac baroreceptors (in the walls of the atria and the ventricles) and the arterial chemoreceptors. Collectively they provide moment to moment information about the pressures in the arterial system, cardiac chambers and great veins as well as the chemical composition of the arterial blood. The NTS is the primary site of reflex integration for regulation of cardiovascular homeostasis (Spyer 1984), (Machado, Mauad et al. 1997), (Pei, Braak et al. 2001), (Davis, Derbenev et al. 2004). It is located within the medulla in the lower brainstem. The arterial baroreceptor afferents terminate in the NTS. It is thought that the baroreceptor reflex arc is not a simple reflex arc and involves relays at several levels within the medulla, pons, midbrain and hypothalamus (Spyer 1984).

The efferent limb of the arterial baroreflex is carried in the parasympathetic (vagal) and sympathetic nerve supply to the heart and vasculature. The cell bodies of the vagal cardiac preganglionic motor neurones are located in the NA and in the dorsal vagal motor nucleus (DVMN) (McAllen and Spyer 1976). The sympathetic preganglionic cell
bodies are located in the IML columns of the thoracic and upper lumbar segments of the spinal cord (Henry and Calaresu 1972).

1.2.2.2 Efferent Medullary Baroreflex Nuclei

Baroreflex fibres from the NTS cross the Cardiovascular Reflex Arc (CRA) to the NA, the dorsal vagal motor nucleus (DVMN) which mediate the efferent baroreflex parasympathetic responses (McAllen and Spyer 1976), (Chitravanshi and Calaresu 1992). Excitatory neurones project from the NTS to the CVLM (Pilowsky 2002). Inhibitory inter-neurones arise from the CVLM and project to the RVLM which mediates the sympathetic baroreflex responses respectively (Pilowsky 2002).

1.2.2.2.1 Vagal Efferent Limb of the Baroreflex.

Two populations of vagal preganglionic neurones have been identified namely the DVMN and the NA which project to the ganglion located near the cardiac pacemaker. They receive a projection from the NTS which in turn receives baroreceptor afferent projections. Baroreceptor afferent activity appears to influence vagal efferent activity via two main pathways: a short latency pathway, which is complete within the medulla, i.e. a segmental pathway travelling from the NTS to the NA, and a longer latency pathway which ascends to relay in the anterior hypothalamus before descending to the NA. Activation of either of these pathways following stimulation of the baroreceptors leads to excitation of the vagal cardiac preganglionic motor neurones within the NA and consequently to bradycardia. The NA is considered the primary medullary nucleus responsible for heart rate regulation, containing cardiovagal fibres which innervate the sino-atrial node of the heart (Chen and Chai 1976), (Corbett, Saha et al. 2003).
1.2.2.2  Sympathetic Efferent Limb of the Baroreflex.

The cell bodies of the sympathetic preganglionic neurones (SPNs) are found exclusively in the spinal cord. The neurones which project to the heart are located in the upper segment of the thoracic spinal cord (T1-5), and the remainder are distributed throughout the thoracic and upper lumbar segments of the spinal cord. They are located in four topographically distinct nuclei within the intermediate spinal grey matter: the IML, the lateral funicular area, intercalated cell group and the central autonomic nucleus. The most prominent division resides in the IML.

There is a complex topographical organisation of the SPNs, with vasomotor and non-vasomotor functions (Dampney 1994). The diversity in anatomical differentiation of the SPNs suggests that it may be possible for afferents inputs from several levels of the neuroaxis to selectively recruit specific preganglionic motor neurones by selective release of specific neurotransmitters (Dampney 1994).

The RVLM provides tonic sympathetic activation to maintain vascular tone (Ross, Ruggiero et al. 1984), (Madden, Stocker et al. 2006). The sympathetic preganglionic neurones (SPN) are controlled by medullospinal sympathoexcitatory neurones located in the RVLM and the rostral ventromedial medulla (RVMM). These sympathoexcitatory neurons are inhibited by baroreceptor interneurones whose cell bodies are located in the caudal ventrolateral medulla (CVLM). Unloading of the arterial baroreceptors results in reduced neurotransmitter release in the NTS which leads to reduced firing of the excitatory neurones that project from the NTS to the CVLM (Pilowsky 2002). Inhibitory inter-neurones arise from the CVLM and project to
the RVLM. Reduced firing of these inhibitory neurones will increase firing of tonically active presympathetic neurones in the RVLM, so increasing sympathetic vasomotor drive (Dampney 1994). Therefore the control of SPN occurs through a process of ‘disinhibition’ (Dampney 1994), (Pilowsky 2002). Efferent sympathetic fibres reach the heart from ganglionic cells of the paravertebral chain and parasympathetic post-ganglionic fibres originate from the cardiac plexus (Gabella 1976). The combination of excitation of the cardiac parasympathetic and inhibition of the cardiac sympathetic fibres produces the bradycardic response of the baroreflex (Figure 3).

It is suggested that there is a discrete region, an intermediate nucleus between the NTS and RVLM, which is responsible for the depressor limb of the baroreflex; i.e., which inhibits the excitation of RVLM neurons. The RVLM contains a mixture of excitatory noradrenergic (A₁) (Day, Ro et al. 1983) and inhibitory adrenergic (C₁) neurons (Benarroch, Granata et al. 1986), (Blessing 1988). Inhibition of the RVLM is associated with the ‘vasodepressor baroreflex response’ (Benarroch, Granata et al. 1986), (Machado, Mauad et al. 1997). Stimulation of the RVLM triggers the adrenal gland to secrete nor-adrenaline to the plasma, which subsequently activates vascular α₁-adrenoreceptors which induces constriction of the smooth muscle cells in arteries and veins thus increasing peripheral resistance (Appenzeller and Oribe 1997).
Carotid Sinus and Aortic Arch baroreceptors activate the baroreflex, transmitted via Vagal (X) and Glossopharyngeal (IX) fibres to brainstem nuclei in the medulla. The reflex enters the NTS, travels to the NA and CVLM which regulates the parasympathetic limb and sympathetic limbs of the baroreflex on the cardiac ganglion and peripheral resistance vessels. Regulation of sympathetic activity is by a process of disinhibition of the inhibitory interneurons which travel from the CVLM to the RVLM. The RVLM provides excitatory fibres to the SPNs in the IML.
Alteration of homeostatic reflex function by soft tissue injury ('injury') has been recognised for many years (Wang 1947), (Overman 1947). Overman and Wang were the first to suggest that nociceptive afferent impulses arising from damaged tissues were important in determining survival after injury.

Soft tissue injury is associated with a different cardiovascular response to that seen with haemorrhage. 'Injury' results in an increase in arterial blood pressure accompanied by a tachycardia. This increase in arterial pressure is largely mediated by an increase in sympathetic activity (Redfern 1981). Normally an increase in arterial pressure would be expected to result in a reflex-mediated reduction in HR as a result of the negative feedback baroreflex response. However 'injury' is associated with a reduction in baroreflex sensitivity and rightward re-setting of the baroreflex, towards a relative tachycardia (Little, Randall et al. 1984), (Jones 1989), (Anderson, Little et al. 1990).

The afferent pathway of this response runs in somatic fibres (including nociceptive fibres) arising from damaged tissues, to the brain via the spinothalamic tracts. The central nervous system organisation of the response to 'injury' is poorly understood. There is, however, a significant body of evidence regarding pathways which modulate the cardiovascular function and the defence response. The 'visceral alerting' response of the defence response has been implicated in the response to 'injury' (Lovick 1992), as the cardiovascular response elicited by 'injury' is very similar to the defence response (Overman 1947), (Hilton 1982), (Redfern, Little et al. 1984), (Coote 2007). This reaction involves a centrally coordinated pattern of behavioural and cardiovascular
responses to prepare the host to meet the challenge imposed by danger. The cardiovascular responses in this preparation for fight or flight include an increase in both heart rate and arterial blood pressure sustained by an inhibition of the arterial baroreflex (Quest and Gebber 1972). There is also a selective increase in skeletal muscle blood flow, presumably to support the anticipated increase in activity (Timms 1981), (Hilton 1982).

Soft tissue injury is also associated with an increased arterial pressure, tachycardia, increased cardiac output, widespread vasoconstriction and increased blood flow to skeletal muscle, which occurs at the expense of blood flow to skin, kidney and the intestine (Little, Yates et al. 1980), (Mackway-Jones, Foex et al. 1999). ‘Injury’ and the defence response also have similar effects on the baroreflex, resulting in a reduction in sensitivity and a rightward re-setting of the baroreflex, i.e. towards a relative tachycardia (Quest and Gebber 1972), (Redfern, Little et al. 1984), (Jones, Kirkman et al. 1990), (Anderson, Little et al. 1990).

The principal brain regions which integrate the autonomic and behavioural components of the defence response are the hypothalamus and the dorsomedial periaqueductal grey (PAG) matter. PAG has been shown to display increased neuronal activity after injury (Jones 1989). Also lesions in the periaqueductal grey prevent the inhibition of the baroreflex elicited by ‘injury’ (Jones, Kirkman et al. 1990). The full pattern of cardiovascular changes associated with the defence reaction can only be elicited from the dorsomedial PAG matter of the midbrain (Hilton and Redfern 1986), (Bandler and Carrive 1988), (Bandler, Carrive et al. 1991). Four longitudinal columns of PAG have described: dorsomedial, dorsolateral, lateral and ventrolateral (Carrive, Bandler et al.
1989). The dorsomedial and dorsolateral PAG are largely pressor regions, whilst the lateral and ventrolateral PAG are depressor (Lovick 1985). PAG is thought to contain groups of cells which are viscerotopically organised with respect to control over various vascular beds (Carrive, Bandler et al. 1989). These fibres project to the RVLM, which in turn also demonstrates viscerotopical organisation (Dampney 1994). Thus PAG has both the anatomical and functional organisation required to orchestrate the complex integration needed to produce the highly specific cardiovascular modulation triggered by the defence response (Figure 4).

1.2.3.1 'Injury-induced' modification of vagal efferent activity

The ‘injury’ induced tachycardia is mainly due to the reduction in vagal activity the heart (Redfern, Little et al. 1984). Under normal conditions when the baroreceptors are stimulated activity in the vagal cardiac motoneurones increases via two main excitatory pathways: from the NTS there is both a segmental pathway within the medulla and a pathway ascending to relay in the anterior hypothalamus before descending again to the vagal nuclei. The defence pathways modulate the baroreflex elicited vagal activity by at least three mechanisms:

1. inhibitory GABA-ergic projections from PAG onto the vagal cardiac motoneurones (Jordon 1980).
2. inhibition within the NTS (McAllen and Spyer 1976).
3. activation of the defence reaction can lead to an excitation of inspiratory neurones within the NA which will inhibit the vagal cardiac preganglionic motoneurones (Spyer 1984).
1.2.3.2 ‘Injury-induced’ modification of sympathetic activity

The pressor response to ‘injury’ is unaffected by cardiac autonomic blockade but abolished by the α-adrenoreceptor antagonist phentolamine (Redfern 1981). ‘Injury’ is associated with an increase in sympathetically induced total peripheral vascular resistance (Overman 1947), (Little, Yates et al. 1980), (Foex, Kirkman et al. 2004). This response is associated with a selective activation of sympathetic activity in different vascular beds, resulting in a highly organised pattern of increased resistance in renal and mesenteric vascular beds and a reduction in resistance in the skeletal muscle vasculature. There is topographical differentiation within the PAG in which this response is highly coordinated (Carrive, Bandler et al. 1989).

The efferent pathways from PAG mediating the cardiovascular effects relay in the nucleus PGL in the rostral ventrolateral medulla (Hilton, Marshall et al. 1983). The PGL appears to integrate the efferent activity of a number of the cardiovascular reflexes and response patterns of the defence response. It receives inputs from the hypothalamic and PAG defence areas, the NTS, the CVLM, the nucleus raphe magnus (NRM) and obscuries (NRO) and the nucleus parabrachialis (Lovick 1988). The PGL, in turn sends and efferent output to the IML and the dorsal horn of the spinal cord (modulation of nociception), (Martin, Humbertson et al. 1979). Stimulation within PGL therefore produces sympathetic excitation and antinociception (Lovick 1987).

There are a number of central nervous pathways which appear to be involved with the cardiovascular response to ‘injury’. The full detail of these pathways has not been fully elucidated at this time.
Figure 4. Schematic representation of the major afferent and efferent pathways of the rostral ventrolateral medulla (RVLM) region in the brainstem.

Major afferent and efferent connections of the rostral ventrolateral medulla (RVLM) region containing pre-sympathetic vasomotor fibres to the intermediolateral cell column (IML). These connections include connections from the lateral hypothalamus (LHA) and paraventricular nucleus (PVN) as well as the periaqueductal grey matter (PAG) which subserve cardiovascular reflexes as well as cardiovascular changes associated with complex behavioural responses. (KF) Kölliker-Fuse nucleus, which is thought to play a role in the neuroendocrine response to blood loss.
1.2.3.3 The cardiovascular response to combined haemorrhage and tissue injury.

The bi-phasic heart rate and blood pressure response to haemorrhage is significantly altered by the presence of soft tissue injury. ‘Injury’ is associated with an increase in blood pressure and heart rate. ‘Injury’ induced tachycardia is largely mediated by an increase in sympathetic activity resulting in vasoconstriction and an increase in peripheral vascular resistance (Redfern 1981). There is a reduction in baroreceptor sensitivity and a rightward re-setting towards a tachycardia (Redfern 1981). This occurs in man within 3 hours of injury and persists for up to 14 days post injury (Anderson, Little et al. 1990). [See section 1.2.3.1 for detailed discussion].

In addition to the modification of baroreflex activity, ‘injury’ is also associated with an attenuation of the bradycardia and hypotension associated with phase 2 of the bi-phasic response to simple haemorrhage (Little, Marshall et al. 1989). The prevention of vagal bradycardia as blood loss progresses is considered to be the result of a central inhibition of vagal cardiac pre-ganglionic motor neurones in the NA (Wang and Li 1988). Blood pressure and heart rate are maintained close to normal values for longer when haemorrhage occurs in the presence of ‘injury’. However, this apparent improved tolerance of haemorrhage is potentially misleading as there is evidence that mortality is significantly higher when haemorrhage co-exists with injury (Wang 1947), (Rady, Little et al. 1991).

Acute TBI has a potentially wide ranging impact on cardiovascular control mechanisms, depending on the location and extent of neuronal injury. TBI is a common injury in multiple trauma patients (McMahon, Yates et al. 1999), (Patel, Bouamra et al. 24
2005). Existing laboratory evidence suggests that cardiovascular compensation for acute blood loss is altered in the presence of acute TBI (Yuan, Wade et al. 1991) (Yuan and Wade 1992), (Law, Hovda et al. 1996). This could be an important factor contributing to the additional mortality seen when acute TBI co-exists with extracranial injury (Gennarelli, Champion et al. 1994), (McMahon, Yates et al. 1999), (Patel, Bouamra et al. 2005). The effect(s) of acute TBI on acute cardiovascular homeostasis is poorly understood.
1.3 TRAUMATIC BRAIN INJURY

There are two broad categories of neuronal damage following TBI:

**Primary brain injury** occurs at the moment of injury (cerebral contusions, lacerations of the brain, diffuse axonal injury and intracranial haemorrhage). The overall incidence of primary TBI can only be reduced with effective implementation of primary preventative strategies.

**Secondary brain injury** results from activation of a number pathological cascades commencing at the time of injury and continuing for weeks, months or years after the primary insult. Secondary brain injury is produced by a complex network of interacting structural, functional, cellular and molecular changes, including breakdown of the blood brain barrier (BBB), disturbance in cerebral autoregulation, altered metabolism, changes in cerebral perfusion, loss of ionic homeostasis, activation of autodestructive neurochemicals and enzymes, generation of free radicals and genomic changes. Some of these pathophysiological mechanisms of secondary brain injury account for the enhanced vulnerability of injured neuronal tissue to ischaemic insults (DeWitt, Jenkins et al. 1995), which are likely to be more prevalent in the presence of impaired cardiovascular homeostasis.
1.3.1 Primary Brain Injury

Brain injury results from a combination of different types of forces acting on this viscoelastic organ. Static loading occurs when forces are applied to the head gradually over a slow time course (greater than 200ms). Sufficient force of this type results in eggshell fractures of the skull. If the force is sufficiently high this will also result in deformation of the underlying brain structures.

The most common type of mechanical impact to the head is dynamic loading (in less than 200ms, in most instances less than 20ms). This is similar to the loading of the lateral fluid percussion model of TBI. Both of these mechanisms result in ‘tissue strain’ injury (also termed ‘tissue “stress”) to neuronal tissue. Tissue strain can lead to shearing of tissues and cellular elements. It also produces pressure gradients disruptive to brain tissue. Three types of brain injury can result from ‘tissue strain’: cortical contusions in the grey mater nearest the skull, diffuse axonal injury (DAI) in the deeper cerebral white mater injury, where “shear-strain” forces result in damage to the axonal structures, damage to the deep grey matter and axonal tracts in the midbrain (Gennarelli 1993).

Dynamic loading may be of either impact or impulsive type. An example of impact loading is a blow to the head with a blunt object. This results in a combination of contact and inertial forces, the extent of which will be determined by the applied mechanical force. Impulsive injury is by far the most common mechanism of primary brain injury. Impulsive dynamic loading is when the head is set into motion or when the moving head is stopped without it striking anything or is arrested by impact. This is
typical of acceleration/deceleration type injuries. The resulting brain injury is from a combination of inertial forces including translation, rotation and angulation depending on the manner in which the head is moved. Inertial injury produces a non-uniform distribution of pressure and tissue strains that result in the primary injury. Intracranial pressure changes and brain motion due to translational acceleration have been linked to specific focal lesions such as coup and contrecoup contusions, intracerebral and/or subdural haematomas, and brainstem lesions. These strains are exaggerated in all regions where abrupt changes in material and structural properties exist, such as the dural linings and the bony buttresses of the inner skull (sphenoid wing) (Ommaya 1974). Other lesions such as diffuse axonal injury and gliding contusions are more related to rotational acceleration forces (Graham, McIntosh et al. 2000)

An important consideration about primary brain injury is that much of the neuronal damage incurred at the time of the primary injury is cell damage, rather than complete destruction. Injured cells are more vulnerable to secondary insults such as hypoxia and hypovolaemia (DeWitt, Jenkins et al. 1995), (Bramlett, Dietrich et al. 1999), (Matsushita, Bramlett et al. 2001), (Chi, Knudson et al. 2006). Optimal resuscitation and the avoidance of further insults to injured neuronal tissues are essential to minimising the extent of neurological damage following acute TBI.
1.3.2 SECONDARY BRAIN INJURY

The secondary brain injury can be broadly classified into two categories: intrinsic and extrinsic. Over the past decade a large body of research on the molecular, vascular and inflammatory aspects of TBI have improved our understanding of the underlying factors that contribute to secondary brain injury. Our knowledge of this complex pathophysiological area remains incomplete. In this section I have presented an overview of the current literature on secondary brain injury, with particular emphasis on the areas of relevance to the studies undertaken in this project.

Table 1. Factors in secondary brain injury.

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<tr>
<th>Factors in Secondary Brain Injury</th>
<th>Intrinsic factors</th>
<th>Extrinsic factors</th>
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<tr>
<td>Elevated intracranial pressure</td>
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<td>Hypoxia</td>
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<td>Low cerebral blood flow</td>
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<td>Low cerebral perfusion pressure</td>
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<td>Hyperthermia/Hypothermia</td>
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<td>Disruption of BBB</td>
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<td>Altered metabolism</td>
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1.3.2.1 Secondary Brain Injury Intrinsic Factors

Cascades of pathophysiological mechanisms are triggered following primary brain injury. This is the result of trauma-induced neurochemical and biomolecular mechanisms that result in alterations in cerebral blood flow (CBF), disturbance in cerebral autoregulation, alterations in the blood brain barrier (BBB), alterations in cerebral metabolism, disturbance in ion homeostasis and inflammatory responses (Yuan, Prough et al. 1988), (Bouma 1991), (Trevisani, Shackford et al. 1994), (Junger, Newell et al. 1997), (Lenzlinger, Hans et al. 2001), (Morganti-Kossmann, Rancan et al. 2001), (Morganti-Kossmann, Rancan et al. 2002). As a consequence of these secondary pathophysiological mechanisms, injured neuronal tissue is highly susceptible to additional insults such as hypotension and hypoxia (DeWitt, Jenkins et al. 1995), (Bramlett, Dietrich et al. 1999), (Matsushita, Bramlett et al. 2001), (Chi, Knudson et al. 2006). As this phenomenon is now widely accepted, international practice guidelines have been established which are directed at reducing the occurrence of avoidable secondary insults (Bullock, Chesnut et al. 1996), (Maas, Dearden et al. 1997). Despite this focused approach in the care of brain injured patients, hypotensive episodes continue to complicate the early post injury phase (Jones, Andrews et al. 1994), (Jeremitsky, Omert et al. 2003).

1.3.2.1.1 Cerebral autoregulation

Under normal conditions a number of protective physiological mechanisms operate to optimise global cerebral circulation and ensure regional CBF is matched with the metabolic needs of neuronal tissue. The capacity of the cerebral vasculature to change
vascular resistance in response to alterations in local and systemic physiological parameters has been recognised since 1890, when Roy and Sherrington stated that:

"the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations in functional capacity" (Roy 1890)

CBF is regulated by a process of cerebral autoregulation, which functions to maintain CBF at a more or less constant level over a range of MAP of 50-150mmHg (Lassen 1959). Changes in blood vessel calibre mainly take place in the 'cerebral resistance vessels' (i.e. arterioles with a diameter of 30 to 300 μm) (Kontos, Wei et al. 1978). The mechanisms that mediate autoregulation include: the myogenic response of cerebral vessels, metabolic factors, neural mechanisms and activation of potassium channels (Kontos, Wei et al. 1978), (DeWitt, Yuan et al. 1988).

Cerebral autoregulation is disrupted following severe TBI (Overgaard 1974), (Cold 1990), (Golding, Robertson et al. 1999), (Golding 2002), (Schmidt, Czosnyka et al. 2003). Disturbance in cerebral autoregulation has also been shown to complicate even mild TBI (Strebel, Lam et al. 1997), (Junger, Newell et al. 1997) and is more pronounced on the side of injury (Schmidt, Czosnyka et al. 2003). The cause of failure of cerebral autoregulation is unclear, but it is postulated that damage of the endothelial cells of the cerebral vessels may contribute to this disturbance (McIntosh, Smith et al. 1996). Impaired cerebral autoregulation contributes to the increased vulnerability of the injured brain to secondary hypotensive insults (Chesnut, Marshall et al. 1993), (DeWitt, Jenkins et al. 1995), (Cherian, Robertson et al. 1996), (Bramlett, Dietrich et al. 1999), (Jeremitsky, Omert et al. 2003).
CBF increases in the injured cortex to over 100% of baseline (Muir, Boerschel et al. 1992), and then falls to 40-50% of baseline within 15-30 minutes after injury (Yuan, Prough et al. 1988) (Muir, Boerschel et al. 1992). This lasts many hours after injury and is associated with uncoupling of cerebral glucose metabolism (Hovda, Lee et al. 1995). At least part of the low CBF observed during this period is a consequence of a decreased cerebral perfusion pressure (CPP). CPP is not low enough, however, to explain the 50% reduction in CBF that occurs. CBF remains low even after restoration of BP to normal (Marion, Darby et al. 1991), (Zhuang, Schmoker et al. 1992). This reduced CBF complicating acute TBI leaves the brain particularly vulnerable to oxidative stress because of its high oxygen consumption. The brain has low levels of antioxidant enzymes and high levels of substrates for oxidative reaction (iron, membrane polyunsaturated fatty acids). TBI disturbs the oxygen supply and energy metabolism of the brain resulting in the generation of free radicals in the brain tissue. Free radicals interact with all molecules of the cell causing damage to cellular elements (Rose, Huerbin et al. 2002)

1.3.2.1.2 Blood Brain Barrier

The function of the intact BBB is to allow diffusion of small molecules such as oxygen, carbon dioxide and essential nutrients, while excluding large proteins from the interstitial space of the central nervous system. The BBB is comprised of three cellular components: the endothelial cells, the pericytes and the astrocytes. Cerebral capillaries are characterised by a lack of fenestrations and by abundant tight junctions (zonulae occludentes) between the endothelial cells of the capillary. Vasoactive substances from the blood can only exert their effects on cerebral blood vessels if they act via receptors
on the luminal side of the endothelium or if they are physically transported across the barrier. This is an important protective mechanism for the central nervous system.

There are three distinct cellular layers in the cerebral artery. The *tunica intima* comprises a single layer of endothelial cells lining the vessel lumen, surrounded by a basement membrane which divides to enclose pericytes. The *tunica media* contains the vascular smooth muscle cells (VSM). The VSM mediate the ‘myogenic’ response of the cerebral vessels: the vessel first dilates and then constricts in response to the stretch (an increase in intraluminal pressure) in an attempt to return to its original diameter. The *tunica adventitia* comprises the outermost layer of the cerebral vasculature and includes terminal nerve fibres and connective tissue, which contains fibroblasts and tissue macrophages. There is evidence that the adventitia also plays an important role in regulating vascular function. The astrocytes, whose endfeet ensheath arterioles and capillaries, release a variety of neurogenic substances. These substances diffuse into the tunica media and act on receptors on the VSM to cause either a constriction or dilation. In this way neuronal activity can be tightly coupled to local CBF a phenomenon known as ‘neuro-vascular’ coupling.

The vascular endothelium plays an important role in maintaining the normal physiological function of the cerebral blood vessel wall by releasing relaxing and contracting factors and therefore is an important site of autoregulation. Factors that elicit constriction are known as endothelium-derived contracting factors (EDCFs), and can mediate the responses to many vasoactive stimuli including nor-epinephrine, prostaglandin H₂ and physical forces such as stretch and pressure. The counterbalancing factors are known as endothelium-derived relaxing factors (EDRFs), and elicit dilation.
The function of the EDRFs is to inhibit platelet and leukocyte adherence and accumulation as well as to counterbalance the constriction of the cerebral vessels by locally acting vasoconstrictor substances such as nor-epinephrine, serotonin and prostaglandins originating from circulating blood. The delicate balance of 'neuro-coupling' that is achieved by the activities of the endothelial cell layer is adversely affected by primary TBI which causes injury to the endothelial cells resulting in local disturbance in autoregulation and breakdown of the BBB (Povlishock, Becker et al. 1978), (Wei, Dietrich et al. 1980), (Maxwell, Irvine et al. 1988), (Mathew, Graham et al. 1994), (Marmarou 2003).

Breakdown of the BBB is a hallmark of brain injury pathophysiology (Hicks, Baldwin et al. 1997). This results in extravasation of blood components including red blood cells, polymorphonuclear leukocytes (PMNs) and plasma proteins into the interstitial space resulting in the development of vasogenic oedema. In addition to the disturbance in the BBB, injury to the endothelial cells also results in metabolic problems as glucose is not transported across the cell as it normally would be and a situation of 'metabolic-uncoupling' ensues.

Breakdown of BBB appears to be maximal around the sites of contusions in human studies (Barzo, Marmarou et al. 1996), (Marmarou, Portella et al. 2000), (Marmarou, Fatouros et al. 2000). The extent of disruption of the BBB following acute TBI is significantly increased following the occurrence of secondary insult factors such as hypoxia or hypotension (Beaumont, Marmarou et al. 2002).
Inflammatory responses to acute TBI

Historically the central nervous system was considered an ‘immunologically privileged’ organ as a result of its separation from the peripheral circulation by the BBB. However research over the past two decades has shown that the central nervous system is a rich source of inflammatory mediators. Resident cells of the brain, such as neurones, astrocytes and microglia have been shown to be capable of synthesizing all immune mediators of the peripheral immune system. It is generally accepted that a potent immune response is induced following primary brain injury. The neuroinflammatory response appears to have a dual role which is both beneficial and potentially detrimental to neurological recovery (Rothwell 1996), (Schmidt, Heyde et al. 2005).

Activation of the complement cascade is thought to be one of the first cascades of neuroinflammation activated following acute TBI (Ember and Hugli 1997). This results in complement-mediated induction of BBB dysfunction, and complement-mediated early intracranial invasion by neutrophils and later by monocytes and macrophages due to the release of free oxygen radicals, proteases and complement-mediated induction of pro-inflammatory cytokine synthesis such as tumour necrosis factor (TNF), interleukin (1L)-1β and 1L-6 (Ember and Hugli 1997), (Schmidt, Heyde et al. 2005).

The initial neuroinflammatory cascades result in a number of changes in the injured brain tissue which include not only dysfunction of the BBB, but also brain oedema, chemotaxis, phagocytosis, B-cell activation, necrosis and apoptosis. As the second reparative phase dominates the functions of regenerative astrogliosis, accumulation of
progenitor cells and neuroprotection become the dominant effects of the neuroinflammatory response to primary brain injury (Schmidt, Heyde et al. 2005).

The occurrence of secondary ischemic insult factors in the early stages of this intense neuro-inflammatory response is likely to be associated with an increase in activity of the neuro-inflammatory responses which may contribute to the poorer outcomes seen in this group. There is also evidence that when TBI co-exists with extracranial injury there is an augmentation of the systemic inflammatory response (Seekamp 2002). This is associated with a higher prevalence of subsequent multiple organ dysfunction, longer duration of intensive care support and mortality (Seekamp 2002). Therefore the pathophysiological responses to central nervous system injury are closely influenced by extracranial insult factors and the presence of central nervous system injury also affects the responses to peripheral injury.

1.3.2.1.4 Metabolic changes following acute TBI

The trauma-induced metabolic cascade is characterised by an acute period of hyperglycolysis followed by a chronic state of metabolic depression (Bouma 1991), (Ginsberg, Zhao et al. 1997), (Bergsneider, Hovda et al. 1997). CBF is reduced following injury resulting in a state of compromised substrate delivery and metabolic coupling (Bouma, Muizelaar et al. 1991). The initial high glucose utilisation is thought to be a direct response to the disruption of ionic gradients across the neuronal cell membrane (from action potentials, seizures, spreading depressions). This results in activation of energy-dependent ionic pumps requiring an increase in glucose utilisation (Rosenthal, LaManna et al. 1979). However the mismatch between substrate delivery
and metabolism results in the production of high levels of lactate and the development of intracellular acidosis and neuronal cell injury (Jaggi, Obrist et al. 1990), (Cruz, Hoffstad et al. 1994).

The disruption of the normal ion gradients across cells allows sodium to enter the cell, potassium exits and intracellular calcium increases. Excess intracellular calcium disrupts a number of critical cellular processes including oxidative phosphorylation. High levels of intracellular calcium also results in oxygen radical reactions. Free radicals create a highly unstable environment, that can lead to the release of nitric oxide (NO) and excitatory amino acids such as glutamate. Glutamate acts on the N-methyl-D-aspartate (NMDA) receptor sites causing a further influx of calcium into the cells. Thus the main biomolecular components of cellular injury enhance each other’s activity. Cerebral hypoperfusion and cellular ischaemia all promote oxygen radical reactions thus exacerbating this cycle. If endogenous protective mechanisms, such as free radical scavengers do not halt this cycle, widespread cellular damage or necrosis occurs (Neumar 2000).

1.3.2.2 Extrinsic secondary insult factors

There are a number of extrinsic factors known to adversely affect recovery from primary TBI (Table 1). This discussion is limited to hypoxia and hypotension which are the main negative prognostic factors of TBI outcome and are the factors of relevance to the research undertaken in this thesis.
Over 40% of patients with acute TBI have associated extracranial injuries, with a more frequent occurrence of secondary brain insults (Luerssen, Klauber et al. 1988), (Sarrafzadeh, Peltonen et al. 2001). Secondary ischaemic insults are an important cause of further damage to injured neuronal tissue (Chesnut, Marshall et al. 1993). Poor outcome has been consistently associated with hypotensive or hypoxic episodes complicating TBI (Miller, Sweet et al. 1978), (Miller and Becker 1982), (Luerssen, Klauber et al. 1988), (Pietropaoli, Rogers et al. 1992), (Jones, Andrews et al. 1994), (Chesnut 1995), (Signorini, Andrews et al. 1999), (Sarrafzadeh, Peltonen et al. 2001), (Jeremitsky, Omert et al. 2003). The occurrence of even a brief period of mild hypotension (systolic blood pressure 10-29mmHg lower than normal) is associated with a 50% increase in mortality (Miller 1985), (Luerssen, Klauber et al. 1988), (Chesnut, Marshall et al. 1993). The combination of hypotension and hypoxia increases mortality by 75% (Chesnut 1997).

Many of the pathophysiological responses to primary TBI render injured neuronal tissue more susceptible to extrinsic factors (DeWitt, Jenkins et al. 1995). These risks are likely to be exaggerated early post injury when cerebral ischaemic risk is more pronounced (Bouma, Muizelaar et al. 1991), (Bouma, Muizelaar et al. 1992), (Bouma and Muizelaar 1992), (Bryan, Cherian et al. 1995), (Giri, Krishnappa et al. 2000). Enhanced vulnerability of injured neuronal tissues to hypotensive insults is in part due to the concomitant disruption of cerebral autoregulation (Lewelt, Jenkins et al. 1980), (Schmoker, Zhuang et al. 1992), (DeWitt, Prough et al. 1992), (Matsushita, Bramlett et al. 2001). Laboratory evidence shows that haemorrhagic shock superimposed on TBI impairs cerebral perfusion and that this impairment of cerebral perfusion responds poorly to subsequent resuscitation (DeWitt, Prough et al. 1992), (Matsushita, Bramlett
et al. 2001). Haemorrhagic hypotension increases TBI-induced neuronal damage as reflected by an increase in contusion volume (Matsushita, Bramlett et al. 2001). More severe secondary insults are associated with a greater adverse effect on cerebral perfusion (Fulton, Flynn et al. 1993), (Kita and Marmarou 1994). Hypoxia and hypotension also exacerbate brain oedema in the region of brain contusions by causing increased disruption of the BBB in the region of cerebral contusions (Beaumont, Marmarou et al. 2002).

The negative interaction between extracranial injuries with acute TBI is bi-directional as there is also evidence that acute TBI alters the systemic response to peripheral haemorrhage. This is likely to result in a propensity to secondary ischaemic insults because of impaired systemic cardiovascular homeostasis. There is laboratory evidence that the cardiovascular homeostatic responses to haemorrhagic shock and to resuscitation are impaired following acute TBI (Yuan, Wade et al. 1991), (Fulton, Flynn et al. 1993), (Law, Hovda et al. 1996), (Holtzer, Vigue et al. 2001). It has been postulated that acute TBI and haemorrhagic shock can result in impaired cardiac contractility resulting from a disturbance in catecholamine regulation (Yuan, Wade et al. 1991). However the evidence for this is indirect and has not been substantiated. The catecholamine responses to haemorrhage are exaggerated in the presence of TBI (Yuan, Wade et al. 1991), (Atkinson 2000).

Laboratory data shows that TBI is associated with a reduced ability to maintain vascular tone during the severe stages of shock, suggestive of a possible loss of central nervous system control over the peripheral vasoconstrictor mechanisms (Law, Hovda et al. 1996). The precise pathophysiology of this impaired response to haemorrhage in the
presence of acute TBI is unclear. Secondary insults continue to be a frequent problem for brain injured patients despite the focus on targeted therapies to optimise CPP (Jones, Andrews et al. 1994), (Sarrafzadeh, Peltonen et al. 2001), (Jeremitsky, Omert et al. 2003). This suggests that there may be an acute disturbance of cardiovascular regulation complicating acute TBI which would be detrimental to outcome in the multiple trauma patient with confounding head injury.
1.4 Chapter Summary

Trauma remains one of the leading causes of mortality in Westernised societies (Murray 1997), (Peden 2002). TBI is the injury most commonly associated with death and disability following multiple trauma (Gennarelli, Champion et al. 1989), (Kraus and McArthur 1996), (Patel, Bouamra et al. 2005). Outcome from multiple trauma (Lecky, Woodford et al. 2000) and from isolated TBI (Zink 2001) has greatly improved over the past two decades. This improvement in survival is less evident when acute TBI is a component of multi-system injury (Patel, Bouamra et al. 2005).

Secondary brain ischaemic insults are considered to be the principal cause of the additional mortality observed when TBI is a component of multiple trauma (Chesnut 1993), (Zink 2001), (Jeremitsky, Omert et al. 2003). In support of this hypothesis there is evidence that secondary ischaemic insult factors such as hypotension and hypoxia have a marked adverse effect on outcome (Chesnut 1997), (Jeremitsky, Omert et al. 2003), and ischaemic brain injury is frequently seen in those who die as a result of their injuries (Graham and Adams 1971). Additionally, complex pathophysiological mechanisms are triggered at the time of primary TBI which render injured neuronal tissue more vulnerable to extrinsic secondary insult factors (DeWitt, Jenkins et al. 1995).

Patients with acute TBI frequently sustain co-existing haemorrhage and soft tissue injury (Sauaia, Moore et al. 1995), (Sarrafzadeh, Peltonen et al. 2001). The normal haemodynamic response to hemorrhage is a neurally-mediated bi-phasic HR and BP response (Warren 1945), (Barcroft, Edholm et al. 1944), (Secher, Jacobsen et al. 1992),
(Evans, Ventura et al. 2001). Soft tissue injury significantly modifies the bi-phasic response to haemorrhage (Little, Randall et al. 1984), (Little, Jones et al. 1988), (Anderson, Little et al. 1990). There is also evidence that the arterial baroreflex is depressed following soft tissue injury (Little, Marshall et al. 1989), (Anderson, Little et al. 1990). The effects of acute TBI in the normal response to hemorrhage and the arterial baroreflex are unknown. A better understanding of the effects of acute TBI on homeostatic mechanisms would be an important next step in improving the care of these critically injured patients.
1.5 **CORE AIMS OF THESIS**

1. **Epidemiological study:**
To evaluate the effect of acute TBI of moderate severity on survival of multiple trauma patients, using the trauma database of the UK Trauma Audit Research Network (TARN).

2. **Laboratory Studies:**
   a) To evaluate the effect of acute mild and moderate TBI on the bi-phasic heart rate and blood pressure response to progressive simple haemorrhage.
   b) To evaluate the effect of acute TBI on the performance of Shock Index.
   c) To evaluate the effects of acute mild and moderate TBI on the short term function of the baroreflex.
CHAPTER 2

THE EFFECT OF ACUTE TRAUMATIC BRAIN INJURY OF MODERATE SEVERITY ON MORTALITY AFTER MAJOR TRAUMA

2.1 INTRODUCTION:

There has been a 40% reduction in the odds of dying (adjusted for case mix) after major trauma between 1989 and 1994. This has been attributable to improvements in systems of trauma care which have largely resulted following the recommendations of the Royal Colleges. These recommendations were based on the finding that up to 30% of trauma deaths were potentially preventable (Anderson, Woodford et al. 1988), (Royal, College et al. 1988). The two main causes of potentially preventable deaths following injury were a delay in the recognition of actual or impending hypoxia and delay in the recognition of occult haemorrhage (Anderson, Woodford et al. 1988). This reduction in mortality following major trauma appears to have reached a plateau since 1994 (Lecky, Woodford et al. 2000). Therefore the impact of the introduction of system-wide reform of trauma services appears to have reached its limits. In order to further reduce mortality and morbidity following injury improved knowledge of trauma pathophysiology is essential to enable us to improve the care delivered to these patients.

The current widely respected Advanced Trauma Life Support (ATLS) dogma of trauma care advocates a single approach to all trauma patients (American College of Surgeons, 44
2004). This approach assumes that all homeostatic mechanisms are working normally. There is evidence that this is not the case. Previous work has identified that soft tissue injury results in an early depression of one of the principal homeostatic reflexes responsible for maintenance of blood pressure, the arterial baroreflex (Anderson, Little et al. 1990).

The effect of acute TBI on cardiovascular homeostatic mechanisms is unclear. Acute TBI is known to be complicated by disturbance of cerebral autoregulation (Junger, Newell et al. 1997). It is possible that it is also associated with adverse effects on central regulation of systemic circulation. If this were the case acute TBI of a severity which in isolation would be associated with a low mortality may adversely affect survival when it is combined with extracranial injury.

Preliminary analysis of the UK Trauma Audit and Research Network (TARN) database revealed that 21% of all those who reached hospital alive sustained acute TBI as part of their overall injury but this increased to 64% in those who subsequently died. Some of these deaths were due to the severity of the primary brain injury itself. However acute TBI of moderate severity was disproportionately represented in those who died as a result of their injuries.

In order to test the hypothesis that acute TBI adversely affected outcome from major trauma, a detailed analysis was undertaken using the extensive database of the UK Trauma Audit and Research Network. Factors which are known to affect outcome: age, injury severity, and the presence of pre-existing medical conditions were included in the
analysis (Campbell and Yates), (Baker, O'Neill et al. 1974), (Milzman, Boulanger et al. 1992).

The UK TARN database, a development of the Major Trauma Outcome Study (Yates, Woodford et al. 1992), is the product of a multi-centre prospective cohort study and contains descriptive data on injury severity and outcome after trauma. Inclusion criteria for trauma cases to the database are: admission to hospital for > 72 hours, admission to intensive care or death within 30 days of injury. Isolated neck of femur or single pubic rami fractures in patients over the age of 65 years, and closed isolated limb fractures are not included in the database. At the time of analysis the database contained data on more than 42,000 patients collected between 1989 and 1995 from over 130 hospitals in the UK.

2.2 METHODS:

Injuries were coded using the 1990 revision of the Abbreviated Injury Scale (AIS) (AAA, 1990 revision). Moderate TBI was defined as a single AIS 3 'Internal Organ' injury in the ‘Head Injury’ section of the coding manual. This consists predominantly small cerebral contusions (<30cc; 4cms diameter; < 5mm midline shift) as identified by CT scan, MRI scan or by autopsy. Traumatic subarachnoid haemorrhage, mild degrees of brain swelling were also included in the group (AIS<3). Injuries to the major intracranial vessels (laceration, thrombosis and traumatic aneurysms) are coded separately from internal organ damage. They are also severe injuries and score > AIS 3. They were not included in this study group, as this would have led to the selection of an
overall higher severity of intracranial injury, which was not the subject of this study. Isolated AIS 3 brain injuries were associated with a mortality rate of less than 4.2%.

The study population was limited to patients who sustained blunt trauma with an Injury Severity Score (ISS) between 16 and 50. These parameters were chosen to reflect the range of injury severity where TBI could be expected to affect mortality. The TBI group had an AIS 3 brain injury and extracranial injury contributing to their overall ISS. These patients may have had other associated head injuries with AIS 2 or less (e.g. associated skull fracture). The control group consisted of patients with extracranial injury only. Patients with scalp and skull injuries were not included in the control group in order to avoid the presence of unrecognised associated brain injury in this group. Only cases with complete data were used. This resulted in the exclusion of less than 0.01% of all potential cases.

The probability of death was modelled using logistic regression adjusting for injury severity (ISS), and age. The Revised Trauma Score (RTS) was not used in this study as it is used in TRISS trauma scoring (Boyd, Tolson et al. 1987) because it is heavily dependent on the Glasgow Coma Score (GCS), (see appendix 1). An alteration of GCS in the non-head injured patient is secondary to a different mechanism (e.g. severe hypoxia, hypovolaemia or intoxication). The inclusion of GCS in this analysis would therefore be a potential source of bias. ISS and age together have been shown to be good prognostic indicators of survival (Bull and Dickson 1991). Evidence from the UK TARN database identified that the effect of age on survival was better represented by categories rather than a single step at the age of 55 years as used by TRISS (Campbell and Yates). We therefore used injury severity, age (<16, 16-25, 26-40, 41-55, 56-65,
66-75, 76-85, >85) and the presence of a moderate TBI to analyse the effect of TBI on outcome from multiple trauma. This was achieved using logistic regression in SPSS for Windows version 6 (SPSS 1996).

2.3 RESULTS

There were 378 patients in the moderate TBI and 2,339 patients in the control group. Analysis of the distribution of injuries in both groups revealed an increased incidence of facial injuries in the moderate TBI group. Exclusion of patients with facial injuries did not significantly change the results of the analysis. There were slightly fewer chest and abdominal injuries in the moderate TBI group which could indicate a potential underestimation of the effect of brain injury on outcome as these areas are often associated with significant occult haemorrhage (Sarrafzadeh, Peltonen et al. 2001) (Figure 5; Table 2).

The mortality for an isolated AIS 3 brain injury was 4.2%. Mortality rates for isolated AIS 3 injuries in the other main body regions were also calculated and found not to be significantly lower (AIS 3 chest injury: 3.6%, AIS 3 abdomen injury: 3.2%, p=0.71).

Moderate TBI was associated with an adverse effect on survival throughout the range of injury severity scores (16-50) (Figure 6; Table 3) and ages (Figure 7; Table 4) studied. There appeared to be an additional increased mortality in the 26-40 year age group.

Data on premorbid medical conditions were only available for 40% of patients. Our analysis demonstrated that the confounding effect of pre-existing medical conditions was large. This did not, however, reach statistical significance, the odds ratio adjusted
for ISS 90 and age was 2.18 (95% confidence interval (CI), 0.70 to 6.81). The wide confidence interval is most likely to be secondary to the large amount of missing data (Figure 8; Table 5). The logistic model allows analysis of the independent effect of moderate TBI on the probability of death when other significant variables (ISS, age) are accounted for (Figure 9). This graph demonstrates the additional increase in the probability of death occurring in the presence of moderate TBI, the odds ratio being 2.08 (95% CI, 1.57-2.77). This represents a doubling of the risk of death in patients with moderate TBI as a component of multisystem trauma.

One hundred and seven (28%) of the moderate TBI group and 438 (20%) of the control group were transferred between hospitals for further care. When these patients were excluded from the study the results of the comparisons were unaltered (odds ratio: 2.21, 95% CI, 1.60-3.07).
2.4 DISCUSSION

Acute TBI both with and without extracranial trauma remains the single most important factor contributing to death and disability following accidental injury (Gennarelli, Champion et al. 1994), (Kraus and McArthur 1996), (Murray 1997). Severe TBI obviously has a high associated mortality; however this study suggests that even moderate TBI when combined with extracranial injury has a detrimental effect on survival.

The validity of this study is critically dependent on the analysis of the UK TARN database. As injury severity is a major determinant of outcome it is essential that injury severity is measured accurately. Misclassification may result from either deficiency in the scoring system or inter-rater variation. Injury severity scoring for the UK TARN database is carried out at the North Western Injury Research Centre by a small number of trained staff thereby reducing inter-rater variation (intra-class correlation coefficient between UK TARN staff is 0.97 (95% CI, 0.95 to 0.98) (Campbell MSc thesis 1996)). An underscoring of the severity of these injuries by the Abbreviated Injury Scale could account for the increased mortality associated with the combination of moderate brain injury and extracranial injury. When comparative analysis was conducted for isolated AIS 3 injuries in other main body regions there was no significant variation in outcome.

The multi-centre nature of the study could influence interpretation of the findings if very significant variations in the pattern of patient care were identified in a small group of contributing hospitals. Previous analysis of the UK TARN database has shown a wide spread of Ws scores (Hollis, Yates et al. 1995) (differences between actual and
predicted survival) across hospitals which cannot be explained by either the volume of trauma handled or the presence of a specialist neurosurgical unit. However these differences are spread broadly across the 130 hospitals studied with no centre being sufficiently exceptional to be able to skew the results.

Analysis of outcome of patients injured at different ages demonstrated an apparent increase in mortality in the 26-40 year age groups. This may have been a spurious effect. It is possible however, that alcohol or drug intoxication, well known contributory factors in accidents involving young people, may have contributed to this increase (Yates, Hadfield et al. 1987), (Waller, Stewart et al. 1986), (Zink, Maio et al. 1996; Zink, Schultz et al. 1999). Alcohol is well known to potentiate the effects of trauma on central nervous system damage (Flamm, Demopoulos et al. 1977). The increased incidence of pre-existing medical conditions and associated polypharmacy in the elderly group would result in impairment of normal compensatory mechanisms and therefore be an additional factor affecting outcome. Our findings of an increased mortality in this group is consistent with other work in this area where age has been identified as an independent predictor of poor outcome following acute TBI (Susman, DiRusso et al. 2002), (Kuhne, Ruchholtz et al. 2005), (Franko, Kish et al. 2006), (Thompson, McCormick et al. 2006).

2.4.1 SUMMARY

An association between moderate TBI and increased mortality in multiple trauma patients has been demonstrated. Comparison of appropriately matched populations shows that moderate TBI adversely affects survival following multi-system trauma.
Table 2. Distribution of injuries in patients with/without moderate TBI.

<table>
<thead>
<tr>
<th>Body Areas Injured</th>
<th>Moderate TBI (n=378)</th>
<th>No TBI (n=2339)</th>
<th>$\chi^2$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck</td>
<td>8 (2.1%)</td>
<td>27(1.2%)</td>
<td>2.37(0.124)</td>
</tr>
<tr>
<td>Face</td>
<td>103 (27.2%)</td>
<td>211(9%)</td>
<td>105.8(&lt;0.0001)</td>
</tr>
<tr>
<td>Chest</td>
<td>231 (61.1%)</td>
<td>1585(67.8%)</td>
<td>6.50(0.011)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>93 (24.6%)</td>
<td>847(36.2%)</td>
<td>19.38(0.0001)</td>
</tr>
<tr>
<td>Extremities</td>
<td>305 (80.7%)</td>
<td>1360(58.1%)</td>
<td>69.70(&lt;0.0001)</td>
</tr>
<tr>
<td>Skin</td>
<td>182 (48.1%)</td>
<td>991(42.4%)</td>
<td>4.43(0.035)</td>
</tr>
</tbody>
</table>
Figure 6. Mortality with injury severity in patients with/without moderate TBI.

![Mortality graph with ISS and mortality rates for patients with and without moderate TBI.]

Table 3. Mortality with injury severity in patients with/without moderate TBI.

<table>
<thead>
<tr>
<th>ISS</th>
<th>Moderate TBI (n=378)</th>
<th>No TBI (n=2339)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N)</td>
<td>% Dead</td>
</tr>
<tr>
<td>16-19</td>
<td>166</td>
<td>16.9</td>
</tr>
<tr>
<td>20-24</td>
<td>69</td>
<td>18.8</td>
</tr>
<tr>
<td>25-29</td>
<td>60</td>
<td>38.3</td>
</tr>
<tr>
<td>30-39</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>40-50</td>
<td>33</td>
<td>81.8</td>
</tr>
</tbody>
</table>
Figure 7. Mortality by age group in patients with/without moderate TBI.

Table 4. Mortality by age group in patients with/without moderate TBI.

<table>
<thead>
<tr>
<th>Age</th>
<th>Total (N)</th>
<th>% Dead</th>
<th>Total (N)</th>
<th>% Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;16</td>
<td>52</td>
<td>15.4</td>
<td>125</td>
<td>15.2</td>
</tr>
<tr>
<td>16-25</td>
<td>111</td>
<td>24.3</td>
<td>551</td>
<td>13.4</td>
</tr>
<tr>
<td>26-40</td>
<td>80</td>
<td>36.3</td>
<td>578</td>
<td>13.1</td>
</tr>
<tr>
<td>41-55</td>
<td>51</td>
<td>29.4</td>
<td>471</td>
<td>15.5</td>
</tr>
<tr>
<td>56-65</td>
<td>30</td>
<td>33.3</td>
<td>207</td>
<td>27.5</td>
</tr>
<tr>
<td>66-75</td>
<td>30</td>
<td>46.7</td>
<td>185</td>
<td>35.7</td>
</tr>
<tr>
<td>76-85</td>
<td>16</td>
<td>68.8</td>
<td>167</td>
<td>47.3</td>
</tr>
<tr>
<td>&gt;85</td>
<td>8</td>
<td>87.5</td>
<td>55</td>
<td>60</td>
</tr>
</tbody>
</table>
Figure 8. Mortality with co-existing pre-morbid medical conditions in patients with/without moderate TBI.

Table 5. Mortality with co-existing pre-morbid medical conditions in patients with/without moderate TBI.

<table>
<thead>
<tr>
<th></th>
<th>Moderate TBI (n=378)</th>
<th></th>
<th>No TBI (n=2339)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N)</td>
<td>% Dead</td>
<td>Total (N)</td>
<td>% Dead</td>
</tr>
<tr>
<td>+Pre-Morbid Conditions</td>
<td>46</td>
<td>39.1</td>
<td>480</td>
<td>21.7</td>
</tr>
<tr>
<td>No Pre-Morbid Conditions</td>
<td>62</td>
<td>16.1</td>
<td>478</td>
<td>11.7</td>
</tr>
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</table>
The logistic model allows analysis of the effect of moderate TBI on the probability of death when other variables (ISS and Age) are accounted for. The slope of the graph demonstrates a statistically significant increase in the probability of death due to the presence of moderate TBI. The odds ratio being 2.08 (95% CI, 1.57 - 2.77), doubling the relative risk of dying in the presence of moderate TBI.
CHAPTER 3

THE EFFECT OF ACUTE TRAUMATIC BRAIN INJURY OF MILD
AND MODERATE SEVERITY ON THE BI-PHASIC
HAEMODYNAMIC RESPONSE TO HAEMORRHAGE.

3.1 INTRODUCTION

Acute TBI remains the most important single injury contributing to mortality and
morbidity following multiple trauma (Kraus and McArthur 1996), (Patel, Bouamra et
al. 2005). Analysis of the UK TARN database identified that moderate TBI, which in
isolation is associated with a low mortality rate (<4.7%), is associated with a doubling
of the expected mortality in the multiple trauma patient (McMahon, Yates et al. 1999).

Extracranial haemorrhage and TBI frequently co-exist in multiple trauma victims
(Sauaia, Moore et al. 1995). Clinical and laboratory evidence suggests that TBI
adversely affects cardiovascular compensation for haemorrhage (Yuan and Wade
1992), (Law, Hovda et al. 1996). The precise mechanism(s) underlying these adverse
effects are incompletely understood and further investigation is required if outcomes are
to improve. It has been suggested that failure to recognise or adequately treat
haemorrhage is a contributory factor in 60% of ‘preventable’ trauma deaths, where
there is no TBI (Anderson, Woodford et al. 1988). This is also likely to be a problem in
brain-injured patients, particularly if the acute homeostatic response to haemorrhage is
modified.
Trauma victims commonly suffer at least two extracranial insults: haemorrhage and tissue injury, each of which generates its own pattern of cardiovascular homeostatic reflex response. These interact to yield a third pattern of response (Little, Randall et al. 1984), (Little, Marshall et al. 1989). The initial investigations into the cardiovascular responses to progressive haemorrhage were carried out by Barcroft in the 1940s.

Progressive 'simple' haemorrhage (in the absence of major tissue damage) produces a biphasic HR and MAP response (Barcroft, Edholm et al. 1944), (Secher and Bie 1985). There is an initial tachycardia and increase in vascular resistance while blood pressure is maintained by the baroreflex (phase 1). As haemorrhage continues beyond 20% to 30% of blood volume loss, a second 'depressor' phase becomes apparent which involves a reflex vagally mediated bradycardia, a fall in vascular resistance (secondary to a reduction in sympathetic vascular tone) and hypotension (phase 2) (Barcroft, Edholm et al. 1944). This second phase is not due to a failure of the baroreflex, as it has been shown that at this stage the sensitivity of the baroreflex is increased (Little, Randall et al. 1984). This second phase has been attributed to the recruitment of one or more reflexes (Little, Marshall et al. 1989). However the precise nature of the afferent limb(s) of the depressor reflex remains uncertain. The activity of these reflexes is modified centrally by the presence of nociceptive afferent impulses arising from damaged peripheral tissues, resulting in an attenuation of the bi-phasic response (Anderson, Little et al. 1990), (Turnbull, Kirkman et al. 1993). Blood pressure is therefore maintained higher for longer and the bradycardic phase is attenuated or abolished when haemorrhage occurs in the presence of significant soft tissue injury.

There is experimental evidence that this interaction has a detrimental effect on survival (Overman 1947). [See section 1.2. for more detailed discussion of the pathophysiology of the bi-phasic haemodynamic response to blood loss].
The effect of acute TBI on the neural mechanisms responsible for cardiovascular homeostatic responses to progressive simple haemorrhage is unknown. There is evidence of autonomic uncoupling in patients with severe brain injury (Goldstein, Toweill et al. 1998). However the focus of this investigation was on the effects of mild to moderate TBI where acute functional disturbance of cardiovascular homeostatic function may not be expected. Critical disturbance in cardiovascular regulatory control in the early post injury phase of TBI could have important and unexpected adverse effects on both morbidity and mortality in this group. This disturbance would be reflected in an alteration of the normal bi-phasic response to hemorrhage which is the result of a complex interplay of central nervous system reflexes which are largely coordinated in the brainstem.

This laboratory study was designed to test the effects of acute TBI on the cardiovascular reflex responses to significant simple haemorrhage. Three groups of animals were studied; mild, moderate and control (sham) TBI respectively. Fifteen minutes after brain injury progressive simple haemorrhage was induced over a 20 minute period until 40% of total blood volume was withdrawn. No fluid resuscitation was administered. This controlled experimental model enabled confounding variables such as soft tissue injury, alcohol, age and pre-morbid factors to be excluded. The lateral fluid percussion (LFP) brain injury model delivers reproducible severities of brain injury, allowing investigation of the acute effects of mild and moderate TBI on the cardiovascular reflex response to progressive simple haemorrhage to be undertaken.
3.2 MATERIALS AND METHODS

The study was conducted on terminally anaesthetized male Wistar rats of the Porton strain weighing between 240-260g, kept since weaning on a 12 hours light/dark cycle and fed on Beekay standard rat and mouse diet (B & K Universal, UK). The effects of acute TBI, induced using the LFP injury model (Toulmond, Duval et al. 1993), on the cardiovascular response to haemorrhage were determined.

The present investigation was conducted in accordance with the Animal (Scientific Procedures) Act 1986, which encompasses the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (United States 1996).

3.2.1 SURGICAL PREPARATION

Anaesthesia was induced using isoflurane (Abbott Laboratories, UK; 3% in a 1:1 mixture of $O_2/N_2O$, $FiO_2 =0.5$), in an anaesthetising chamber until cessation of spontaneous movement, loss of the righting reflex, absence of blink reflex and no withdrawal to a noxious stimulus (pinch test applied to the foot). Surgical anaesthesia was maintained using 2-3% isoflurane administered via a mask. The lateral tail vein and ventral tail artery were cannulated using polyethylene cannulae (2FG and 3FG respectively, Portex Limited, UK). Both cannulae were pre-filled with heparinised saline (10IU. ml$^{-1}$ in 0.9% saline). The arterial line was utilised to measure arterial blood pressure using a strain gauge manometer (Sensonor 840, SensoNor, Norway). Electrocardiogram (ECG) needle electrodes were placed subcutaneously on the ventral
surface of the animal to record the electrocardiogram (ECG), from which the HR was measured. A neonatal respiratory monitor was attached externally to the chest wall and connected to a transducer to monitor respiratory movements. Colonic temperature was monitored with a thermocouple inserted 6-8cm past the anal sphincter into the colon and was maintained at $37.8 \pm 0.5^\circ$C (normal temperature for the rat) using a fan, blanket and overhead heating lamp.

On completion of the surgical procedure the isoflurane and $\text{N}_2\text{O}$ were discontinued. Anaesthesia was maintained using continuous intravenous infusion of alphaxalone/alphadalone (Saffan, Pitman-Moore, UK, 18-20mg. Kg$^{-1}$. h$^{-1}$ i.v.) using an infusion pump (Harvard 22, Harvard apparatus, UK). Animals spontaneously breathed oxygen-enriched air. The animal was then transferred to a stereotactic frame for the remainder of the experimental protocol.

3.2.2 LATERAL FLUID PERCUSSION MODEL

A craniotomy was performed stereotactically at the level of the right parietal cortex (3.5mm anterior to and 6mm above the interaural line) leaving the dura intact. Teflon tubing (2mm inner diameter) was placed over the dura and sealed in situ using methylmetacrylate cement. This was connected through a fluid filled system to an HPLC pump (SF400 Spectroflow, Kratos analytical instruments, NJ, USA). The HPLC pump was used to deliver a predetermined level of fluid percussion pressure, which was released onto the lateral cortex using an electronically controlled solenoid valve (RS, UK). Thus a brief pulse (20ms) of sterile water was applied to the surface of the brain at an applied cortical pressure of 1.2atm, producing mild injury and 1.8 atm for moderate
injury respectively (Toulmond, Duval et al. 1993). Control animals underwent identical procedures, but had no applied cortical pressure.

3.2.3 Haemorrhage Model

A fixed volume controlled haemorrhage model was utilised in this study (Majde 2003). [See section 6.33 for discussion on the selection of the haemorrhage model]. Progressive simple haemorrhage was induced by withdrawal of blood from the tail artery anaerobically in 0.5ml aliquots at an overall rate of 2% of the blood volume per minute (total estimated blood volume 6.06ml/100g body weight) until 40% of the total blood volume has been removed (Elebute 1978).

3.2.4 Protocol

A minimum of 60 minutes was allowed to elapse before physiological data were recorded to allow an adequate washout period of isoflurane before commencement of the experimental protocol. The rate of infusion of alphadolone/alphaxolone was adjusted to allow a mild withdrawal and a 10mmHg rise in blood pressure in response to a pinch test applied to the Achilles tendon. The timelines of the experimental protocol are reproduced in (Figure 10).

Experiments were performed on three groups of eight animals. Animals were randomly allocated to mild TBI, moderate TBI or the control (sham) brain injury group. The LFP injury is associated with a brief period of apnoea, hypertension and bradycardia (Gennarelli 1983), (McIntosh, Vink et al. 1989). Therefore to allow physiological
variables to return to baseline before the induction of haemorrhage a further period of 10 minutes was allowed to elapse. Baseline physiological measurements of HR, blood pressure and respiratory rate were then captured as control data for the haemorrhage study. Fifteen minutes following the acute brain injury animals underwent simple hemorrhage of 40% blood volume by methods described. Physiological measurements were made after withdrawal of each aliquot of blood and at 15 minute intervals until 90 minutes post head injury, using a computerized physiological data acquisition system (Fastdaq, Lectromed, UK. Real time data capture, display and analysis system), (Figure 10). Blood gas analysis was subsequently performed on each aliquot (ABL330, Radiometer, Denmark), (Figure 11).

Animals were killed humanely using a lethal injection of Sagital anaesthesia at the end of the post haemorrhage observation period. Post-mortem examination of the brains was carried out on all animals immediately following the procedure.

3.2.5 Statistical Methods

Means and standard errors of HR and MAP are presented by volume of haemorrhage, for control, mild and moderate TBI groups. Baseline data are presented by group using means and standard errors of mean (SEM). Analysis of variance (ANOVA) was used to test for any changes in mean values between the three groups.

To determine if the effects of brain injury and haemorrhage on HR and MAP by volume of haemorrhage are statistically significant, a two-way repeated measure ANOVA was undertaken to examine differences between the groups with repeats for volume of
haemorrhage (12 measurements) within animals. The trend analysis considered 2 models: (1) assuming a linear association of HR and MAP with volume of haemorrhage and (2) assuming a linear and quadratic association. The quadratic time variable allows for any curvature in the relationship between HR or MAP and volume of haemorrhage. A test for interaction of group (injury or control) by volume of haemorrhage (linear and quadratic) was used to determine if there were significant differences in the trend lines between injury and control groups.

In the moderate TBI group, a two-way repeated measures analysis ANOVA was performed to compare the trends for mean HR and MAP (with repeats for different volumes of haemorrhage) comparing survivor and non-survivor animals. A test for interaction was used to examine whether the patterns of response with increasing volume of haemorrhage were different between survivors and non-survivors of moderate TBI. An independent t-test was used to compare mean apnoea between survivors and non-survivors in the moderate TBI group. An animal was classified as a non-survivor if it died within 90 minutes of induction of brain injury (the end point of the study was 90 minutes post induction of brain injury). Significance levels at P<0.05 were assumed. JMP, version 5 and SAS software was used to analyze the data (SAS Institute Inc.)

3.2.5.1 Statistical Power of the Study

With a sample of 8 animals having repeated data on 12 occasions, the study has sufficient power (80%) at a 5% level of significance to detect a difference of 1 SD in HR or MAP between any two groups (e.g. moderate and control) in the study.

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3.3 RESULTS

3.3.1 BASELINE RESULTS

Following acute TBI a brief period of apnoea was observed. There were significant differences between groups in the duration of apnoea induced by the fluid percussion injury. No apnoea was observed in the control group. In mild TBI the duration of apnoea was 3.1 ± 1.1sec compared to 13.6 ± 2.48sec (P< 0.001) following moderate TBI (Table 6).

In mild TBI there was transient elevation of systolic blood pressure of 15 ± 1mmHg compared to 41 ± 6mmHg (p=0.002) in moderate TBI following the fluid percussion injury. No pressor response was observed in sham injury.

There were no significant differences between groups in the initial baseline values of body weight, HR, MAP or arterial blood gases at time control, 10 minutes post acute TBI, which was immediately before the commencement of the haemorrhage phase. HR was observed to be lower in the moderate TBI animals prior to undergoing haemorrhage, but this did not reach statistical significance (Table 6).

Arterial blood gas analysis during the haemorrhage period revealed no significant differences between the groups (Figure 11). The PaCO₂ fell with progressive haemorrhage (linear trend: β=-0.79 (SE=0.10), p<0.001). No hypoxic episodes occurred in the study animals.
Post mortem evaluation of the moderate TBI group revealed macroscopic evidence of contusion on the surface of the ipsilateral cortex with small amounts of subarachnoid haemorrhage as described in other reports (Thompson, Lifshitz et al. 2005). The mild TBI and control animals had no macroscopic evidence of brain injury.

3.3.1.1 Mortality

There was no mortality observed in the control or mild TBI groups. Four (50%) of the moderate TBI animals died suddenly after the completion of haemorrhage, at time 45, 55, 60 and 90 minutes respectively.

3.3.2 Heart Rate and Blood Pressure Responses to Haemorrhage

3.3.2.1 Control group

In the control animals progressive haemorrhage produced a bi-phasic response of HR and MAP (Figure 12). HR showed an initial increase until 17.5 ± 1.6 % blood volume loss occurred. The maximum increase in HR was 24 ± 4.2bpm which indicated a significant change from baseline (p<0.01). However as the severity of haemorrhage increased beyond 17.5% of total blood volume, a marked bradycardia associated with a precipitous fall in MAP took place (Figure 12). The maximum drop in HR was 107 ±7.5 bpm, which indicated a significant change from baseline (p<0.01) and occurred at 38 ± 0.9% blood volume loss.
3.3.2.2 Mild Head Injury Group

The HR and MAP response by volume of haemorrhage was compared between mild TBI and control groups. There was a small delay in the onset of bradycardia. This divergence in response became significant at 27% blood volume loss (p=0.044). However the overall bi-phasic pattern of response was maintained. This was confirmed in the quadratic analysis of the HR and MAP response by volume of haemorrhage. A repeated measures ANOVA showed a significant linear ($\beta_1=14.55$, SE=2.7) and quadratic ($\beta_2=-1.98$, SE=0.21) association of volume of haemorrhage on HR (p<0.0001) for both groups (Figure 12). However the bi-phasic pattern of HR response did not differ significantly between the mild TBI and control groups.

There was a significant quadratic ($\beta_2=-0.61$, SE=0.11) association of volume of haemorrhage on MAP (p<0.0001) for both groups (Figure 12). The largest difference between the mild TBI and control groups was observed at 26.7% volume haemorrhage (difference=55.2 (95% CI, 1.6, 108.8)), but the pattern of MAP response overall did not differ significantly between the mild TBI and control groups. Therefore, whilst the trend of response for HR and MAP was similar in both groups, the depressor phase of the response was delayed in the mild TBI group (Figure 12).
3.3.2.3 Moderate Head Injury

By contrast with mild injury the pattern of HR and MAP response was altered after moderate TBI. Both HR and MAP were initially lower in the pre-haemorrhage phase following moderate TBI. The HR response to haemorrhage in moderate TBI animals was significantly different from that of control animals (Figure 12). The expected biphasic HR response was markedly attenuated after moderate TBI. A divergent pattern of HR response was identified between the moderate TBI and control groups; a higher HR response was observed in the moderate TBI animals (38 ± 8.6 bpm), until 23% volume of haemorrhage was reached when the trend was reversed. The maximum fall in HR was 51 ± 15 bpm at 31% blood volume loss, compared to 107 ± 7.5 bpm in the control group, which occurred at 17.5% blood volume loss. The repeated measures ANOVA showed a significant linear ($\beta_1=10.36$, SE = 2.89) and quadratic ($\beta_2=-1.43$, SE=0.22) trend of volume on mean HR ($p<0.01$). There was a significant difference in this trend of HR response observed between the moderate TBI groups and the control injured group (test for interaction $p<0.001$), and between the moderate and mild TBI groups (test for interaction between groups, $p<0.0001$).

Similarly the MAP response to haemorrhage was also markedly altered following moderate TBI. There was a significant difference in overall linear ($\beta_1=-3.62$, SE=1.47, $p=0.015$) and quadratic trend ($\beta_2=-0.39$, SE=0.11, $p<0.001$), between the groups, across volume of haemorrhage. This indicates that the pattern of blood pressure response to progressive simple haemorrhage was significantly different between the moderate TBI and control groups (test for interaction, $p=0.0007$) (Figure 12) and between the moderate and mild TBI groups (test for interaction between groups,
Overall the MAP was maintained higher for longer in the moderate TBI group compared to either the control group or the mild TBI group.

Although the study was not designed as a survival analysis investigation, following the observed 50% mortality rate in the moderate TBI group a preliminary subgroup analysis of the group was undertaken. Animals were stratified into survivors and non-survivors. Clear differences in the HR and MAP response to haemorrhage between the survivors and non-survivors were identified. Survivors still showed a bi-phasic HR and MAP response which was modified compared to controls, i.e. the onset of bradycardia and hypotension were delayed but the difference was more pronounced than in mild TBI. In non-survivors the bi-phasic HR and MAP response was abolished (Figure 13). At the end of haemorrhage MAP was significantly higher in the non-survivors compared to either the survivors (difference = 42.6, (95% CI, 17.7, 67.5), p=0.007) or the control group (difference = 42.9, (95% CI, 23.4, 62.4), p=0.002).

When HR and MAP responses of non-survivors versus survivors were compared using repeated measures ANOVA there was a significant difference in HR and MAP by volume of haemorrhage (Figure 13), (test for interaction, p=0.0129, p=0.0013 respectively). The mean (SEM) apnoea period in those who died (16.60 ± 2.18 seconds), compared with survivors (10.68 ± 4.27 seconds) was not significantly different (p=0.26). Post mortem evaluation of the moderately injured animals revealed no significant macroscopic difference between animals that died compared to those who survived.

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3.4 DISCUSSION

Assessment and management of the acutely injured patient depends on the assumption that all homeostatic reflexes are functioning normally. The principal finding of this study is that acute TBI modifies the cardiovascular response to haemorrhage and the effect is graded with respect to severity of TBI. After mild TBI the biphasic response to blood loss is maintained, but the onset of the second, depressor phase of the response to haemorrhage is delayed. Moderate TBI significantly attenuated the depressor response to haemorrhage such that MAP was maintained higher for longer in the moderately injured group compared to either the control or the mild TBI group. The effects of acute TBI on the reflex responses to haemorrhage have not been previously elucidated in either man or rodent models.

3.4.1 THE EXPERIMENTAL MODEL

There are several models of haemorrhage used in experimental studies, including controlled blood loss to either fixed volume of fixed pressure endpoints, or uncontrolled haemorrhage, and the choice of model is dictated by the hypothesis being tested (Majde 2003). In the present study the hypothesis being tested was that acute TBI would modify the pattern of response to haemorrhage, consequently the model of choice was a fixed volume, controlled haemorrhage (Majde 2003). This type of model is viewed as the most appropriate when investigating potential modulations of the reflex response to blood loss, where it is essential that the degree and rate of blood loss are the same among experimental groups (Feuerstein and Siren 1986). An uncontrolled haemorrhage model (Dronen, Stern et al. 1993), (Ryan, Cortez et al. 2006) would be inappropriate in
this type of study because the altered pattern of blood loss could introduce a systematic bias between groups. It would become impossible to determine whether any differences in pattern of response to blood loss was due to, or resulted from the difference in profile of blood pressure change during haemorrhage. This investigation was therefore conducted in line with previously published work in this area using fixed volume haemorrhage (Barcroft, Edholm et al. 1944), (Little, Marshall et al. 1989), (Evans, Hayes et al. 1992), (Ludbrook and Ventura 1996), (Ohnishi, Kirkman et al. 1997), (Foex, Kirkman et al. 2004).

In multiple trauma patients extracranial blood loss is often accompanied by soft tissue injury. Soft tissue injury modifies the reflex response to haemorrhage resulting in an attenuation of the phase 2 of the bi-phasic HR and MAP response (Little, Randall et al. 1984), (Anderson, Little et al. 1990), (Foex, Kirkman et al. 2004). In addition, the response to soft tissue injury may interact with the response to TBI to produce further modification of the normal responses. The investigation of a three-way interaction between haemorrhage, TBI and soft tissue injury would be of great clinical importance, interpreting the results would be impossible without prior knowledge of the two-way interaction between the responses to blood loss and acute TBI. The present study has therefore focused on this first step which provides a sound basis on which to investigate models of greater clinical complexity in the future which could ultimately include a number of factors such as co-existing soft tissue injury, the occurrence of secondary insults, intoxication, age and other factors that can influence the reflex responses (Anderson, Little et al. 1990), (Zink and Feustel 1995), (Zink, Schultz et al. 1999).
In considering the selection of the rodent model for this investigation previous work indicates that there is close species correlation in the cardiovascular reflex responses to both haemorrhage and injury in man, in other large animal and in rodent studies (Barcroft, Edholm et al. 1944), (Oberg 1970), (Secher, Sander Jensen et al. 1984), (Secher and Bie 1985).

The choice of anaesthetic used in this model was critically important. Anaesthetic agents can modify the baroreflex and the ‘injury’-induced modulations of baroreflex function. Hence much of the original experimental work carried out on cardiovascular reflex activity was done using conscious animals. We used low dose intravenous alphadolone/alphaxolone anaesthesia as it has been shown to preserve baroreflex function and the effects of ‘injury’ modulations (nociceptive afferent activity) on the baroreflex (Timms 1981), (Mackway-Jones, Foex et al. 1999), (Majde 2003).

The fluid percussion model of brain injury in the rat is one of the most widely used experimental models of brain injury because if its construct validity with closed head injury in man (McIntosh, Vink et al. 1989), (Toulmond, Duval et al. 1993), (Laurer and McIntosh 1999). This model of brain injury enabled reproducible severities of brain injury to be produced. The histological changes resulting from this brain injury model include a well defined ipsilateral cortical injury, ipsilateral hippocampal and amygdaloid injury (Toulmond, Duval et al. 1993), (Thompson, Lifshitz et al. 2005). Other aspects of molecular, vascular and behaviour of different severities of human brain injury are closely replicated in this model (Toulmond, Duval et al. 1993).
3.4.2 Principal Findings.

The biphasic HR and MAP response to haemorrhage observed in the control group was consistent with previous investigations reported in man (Barcroft, Edholm et al. 1944), (Secher, Sander Jensen et al. 1984) and animal studies (Oberg 1970), (Secher and Bie 1985). Following mild TBI the bi-phasic pattern of HR and MAP response was maintained, but the onset of the 'depressor phase' of the response was delayed. No mortality was observed in this group.

In the moderate TBI group the bi-phasic HR and MAP response was markedly attenuated. The HR response to haemorrhage in this group was significantly different to that of both the control and mild TBI groups. The bi-phasic HR and MAP response was markedly attenuated or abolished in this group and the degree of alteration of the response was predictive of early mortality.

These observations are important in the context of what is known about the underlying pathophysiological responses to haemorrhage. In the absence of TBI the neurohumoral and haemodynamic responses to progressive haemorrhage have two distinct phases. There is an initial arterial baroreceptor-mediated phase (Phase 1) in which the fall in cardiac output is coupled with a sympathetically mediated increase in peripheral resistance so that arterial pressure is maintained to near normal levels (Secher and Bie 1985). In most species, adrenal catecholamines and vasopressin contribute little to this phase. Increased renin release appears to augment the sympathetically mediated vasoconstriction. With severe haemorrhage (beyond 25-30% blood volume loss) a second phase (Phase 2) develops abruptly. This phase is characterised by withdrawal of
sympathetic vasoconstrictor drive, relative or absolute bradycardia, which is vagally driven, an increase in adrenal catecholamines and vasopressin, and a profound fall in arterial pressure (Little, Marshall et al. 1989). Acute TBI appears to modify these complex physiological reflex responses to haemorrhage. The precise pathophysiological mechanisms underlying the second ‘depressor’ phase are not fully understood. Part of the efferent limb of the ‘depressor’ response is vagally mediated. This study suggests that acute TBI significantly alters the vagally mediated modification of the HR response normally seen after 20-30% blood volume loss.

A 50% mortality rate was observed in the moderate TBI group within 90 minutes of completion of haemorrhage. Whilst this investigation was conducted as a non-survival analysis, following the observation of such a high early mortality a subgroup analysis of the moderate TBI group was undertaken. Animals were stratified into survivors and non-survivors. Significant differences in the bi-phasic response were observed between these two subgroups. In survivors the bi-phasic response was maintained but the depressor phase was delayed and the difference in response compared to control animals was more pronounced than in mild TBI. In non-survivors the bi-phasic HR and MAP pattern of response was completely abolished. The mechanism(s) underlying the attenuation of haemorrhage-induced bradycardia in the presence of acute TBI cannot be deduced from this study. Similar work evaluating the effect of major tissue damage on the bi-phasic response to haemorrhage also showed attenuation of the response and depression of the baroreflex (Little, Marshall et al. 1989), (Anderson, Little et al. 1990), (Foex, Kirkman et al. 2004). It is possible therefore that baroreflex function may also be altered in the presence of acute TBI.
The moderate TBI group appeared physiologically more ‘stable’ during the initial response to haemorrhage. This was more apparent than real as 50% of this group died suddenly shortly after the completion of haemorrhage. This terminal event was heralded by sudden onset of dysfunctional respiration coupled with bradycardia and hypotension. The precise characterisation of the cause of this sudden death was outside the scope of our study design. A possible explanation might be that brainstem ischemia occurred at that time. Further research to correlate the loci of central nervous system injury with cardiovascular pathophysiology is required to test this hypothesis. Another explanation could be that this subgroup sustained significantly raised ICP, however, an increase in MAP and a bradycardia associated with raised ICP did not occur in this group (Cushing 1902). At the severe end of the brain injury spectrum there is evidence from clinical studies of autonomic uncoupling, which is also likely to reflect severe brainstem injury (Goldstein, Toweill et al. 1998). Disturbance in cardiovascular regulation following acute TBI is further evidenced by work showing that the response to fluid resuscitation following acute TBI and haemorrhage is impaired (Yuan and Wade 1992). Disruptions of the normal cardiovascular homeostatic mechanisms are likely to leave the acute brain injured victim more vulnerable to hypotensive insults which in turn are associated with a significant increase in mortality.

Another complication of acute concussive brain injury observed in both humans and animal models of TBI is transient apnoea (Gennarelli 1983), (Atkinson, Anderson et al. 1998). This appears to be related to ‘brainstem torque’ occurring at the time of injury as it is also observed in decerebrate animal models of brain injury (Tomlinson 1970). An apnoeic response was observed which closely reflected the severity of induced brain injury. This is consistent with other laboratory data (Walker 1944), (Atkinson,
Anderson et al. 1998). Dysfunctional respiration and apnoea occurring at the time of TBI can result in hypoxic episodes. Hypoxia inhibits baroreflex induced bradycardia (Kongo 1999). This was not a factor in our model as supplemental oxygen was delivered throughout the experimental period. This would not therefore account for the observed modulation in the vagally-mediated bradycardia.

Observation of the trend in PaCO₂ levels with progressive haemorrhage showed the expected fall in the PaCO₂ levels in all groups. There was no significant difference observed in the pattern of response between the three groups. Whilst this was not a detailed analysis of chemoreceptor function, it suggests the chemoreceptor response to haemorrhage was maintained in the presence of acute TBI.

3.4.3 LIMITATIONS OF THE STUDY

This investigation was specifically focused on the assessment of the bi-phasic haemodynamic response to haemorrhage. It was not designed as a survival analysis study. This limited the power of the subgroup analysis of survivors and non-survivors. Since this study was conducted as non-survival experiments detailed histological analysis of the injured brains was not performed. This would have required survival of the animals for a number of days (Toulmond, Duval et al. 1993). Post mortem evaluation of the moderately injured animals revealed no macroscopic difference between survivors and non-survivors. The precise cause of death in non-survivors was not accurately determined. They had similar pre-terminal observations: an abrupt onset of a brief period of dysfunctional respiration, bradycardia and hypotension resulting in asystolic cardiac arrest within seconds of onset of dysfunctional respiration. This
laboratory study identifies an acute disturbance in the neural responses to acute haemorrhage in the rodent model using well validated models of closed head injury and simple haemorrhage. Whilst there is evidence of close species correlations in the pathophysiology of TBI and the physiological responses to haemorrhage, it would be important that these findings are validated in the clinical setting.

This investigation was limited to the evaluation of the effect of acute TBI on the bi-phasic haemodynamic response to progressive simple haemorrhage. In the clinical scenario of multi-system trauma, haemorrhage is often associated with major tissue injury (Sarrafzadeh, Peltonen et al. 2001). Further investigation into the effects of acute TBI on the response to haemorrhage confounded by co-existing soft tissue injury is an important next step in improving our understanding of the pathophysiology of acute TBI on cardiovascular regulation.

3.4.4 CONCLUSIONS

Acute TBI significantly modifies the normal cardiovascular reflex responses to progressive simple haemorrhage. This effect is graded according to the severity of the induced brain injury. This modification was observed even in the presence of mild TBI. Moderate TBI was associated with a marked alteration in HR and MAP response to haemorrhage. Acute disturbance in cardiovascular regulatory control potentially has profound implications for trauma victims who sustain combination of acute TBI and haemorrhagic shock. This may in part explain the unexpectedly high mortality rate seen in patients who sustain acute TBI as a component of multi-system trauma (McMahon, Yates et al. 1999), (Patel, Bouamra et al. 2005). Currently there are no readily available
clinical tools to assess the integrity of autonomic function in the acute clinical setting.
This may be a very important factor influencing the approach to resuscitation and choice of anaesthetic agents used in the clinical care of these patients. Further clinical and laboratory research in this area is needed to improve our understanding of the cardiovascular pathophysiology in brain injured patients and thereby translate into improved outcomes in this group.
Table 6. Physiological Measurements: post-TBI and pre-haemorrhage for Control, Mild and Moderate TBI groups.

<table>
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<td>PaO₂ (mmHg)</td>
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<td>264.5 (177.6,351.4)</td>
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<tr>
<td>pH</td>
<td>7.33 (7.31,7.35)</td>
<td>7.34 (7.32,7.35)</td>
<td>7.33 (7.30,7.36)</td>
</tr>
<tr>
<td>Hb</td>
<td>12.38 (12.01,12.74)</td>
<td>11.61 (10.88,12.34)</td>
<td>12.03 (11.72,12.35)</td>
</tr>
<tr>
<td>ABE (mmol.l⁻¹)</td>
<td>0.91 (0.29,1.54)</td>
<td>0.61 (-0.12,1.35)</td>
<td>1.0 (-0.03,2.03)</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>37.69 (37.55,37.83)</td>
<td>37.66 (37.53,37.79)</td>
<td>37.61 (37.52,37.70)</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>254.8 (249.0,260.5)</td>
<td>250.3 (247.2,253.4)</td>
<td>251.4 (246.6,256.2)</td>
</tr>
<tr>
<td>Apnoea sec*</td>
<td>0 (0, 0)</td>
<td>3.1 (0.5,5.6)</td>
<td>13.6 (7.8,19.5)</td>
</tr>
</tbody>
</table>

*p<0.0001 ANOVA between groups

Number of rats (n): arterial oxygen pressure (PaO₂): carbon dioxide tension (PaCO₂): arterial pH (arterial pH): Haemoglobin (Hb): arterial base excess (ABE): core body temperature (Tc): Body weight (body wt): Values are mean (95% Confidence Interval).
Figure 10. Diagrammatic representation of the Study Protocol.
Figure 11. Mean (± SEM) of PaO2, PaCO2, ABE, pH for control, mild and moderate TBI groups with 40% haemorrhage.
Figure 12. Mean (±SE) MAP, HR for increasing % volume of haemorrhage in mild TBI, moderate TBI and control groups. In the moderate TBI group 8 animals were alive at T45; 5 animals alive at T60 and T90 min.
Figure 13. Mean (± SE) MAP and HR for increasing % volume of haemorrhage in the moderate TBI survivors, moderate TBI non-survivors and control groups.
CHAPTER 4

THE EFFECT OF ACUTE TBI OF MILD AND MODERATE SEVERITY ON THE PERFORMANCE OF SHOCK INDEX IN THE PRESENCE OF PROGRESSIVE SIMPLE HAEMORRHAGE

4.1 INTRODUCTION

The early identification of occult haemorrhage in the multiply injured patient can present a difficult clinical challenge. Delay in the recognition and stabilisation of occult haemorrhage is one of the key factors contributing to preventable trauma deaths (Anderson, Woodford et al. 1988). The initial triage and evaluation of injured patients is guided by a combination of factors which include the patient’s heart rate and blood pressure (ATLS, American College of Surgeons, 2004). However vital signs are known to be unreliable early indicators of blood loss (Little, Kirkman et al. 1995).

Progressive ‘simple’ haemorrhage (in the absence of major tissue injury) is associated with a biphasic HR and MAP response (Barcroft, Edholm et al. 1944), (Secher and Bie 1985). This non-linear relationship of HR and MAP with progressive haemorrhage is one of the factors contributing to the difficulty in the interpretation of vital signs in the trauma patient with occult haemorrhage. [See section 1.2 for detailed discussion on the neural mechanisms underlying the cardiovascular response to haemorrhage.] A variety of factors commonly present in the trauma patient can modify the normal neurally mediated bi-phasic haemodynamic response to haemorrhage. These include co-existing soft tissue injury (Overman 1947), (Little, Marshall et al. 1989), (Anderson, Little et al.
and the administration of opioid analgesics and anaesthetic agents, which are commonly used in the care of injured patients (Adamicza, Tarnoky et al. 1985), (Ebert, Kotry et al. 1985), (Van Leeuwen, Evans et al. 1990), (Ohnishi, Kirkman et al. 1997).

In order to overcome the limitations of HR and BP in the recognition of occult haemorrhage Allgower and Buri introduced the Shock Index (SI); the ratio of HR to systolic blood pressure (SBP) (Allgower and Burri 1967). SI has been shown to be a more sensitive indicator of blood volume loss than either HR, SBP, or diastolic blood pressure (DBP) in patients presenting with acute haemorrhage (Allgower and Burri 1967), (Birkhahn, Gaeta et al. 2003), (Birkhahn, Gaeta et al. 2005).

SI has a relatively linear relationship with blood volume loss (Little 1990). This linear relationship is maintained in the presence of soft tissue injury (Little 1990). Acute TBI is commonly present in the multiply injured patient (Gennarelli, Champion et al. 1994), (Patel, Bouamra et al. 2005). Furthermore acute TBI significantly alters the normal biphasic haemodynamic response to simple haemorrhage (McMahon 2007). The performance of SI when extracranial haemorrhage occurs in the presence of acute TBI is unknown. The aim of this study was to examine the relationship between SI and progressive simple haemorrhage in the presence of acute TBI of mild and moderate severity.

4.2 METHODS

The performance of SI in response to progressive extracranial haemorrhage following mild and moderate TBI was assessed using the preclinical models of acute TBI and
controlled simple hemorrhage. [See Methods Section; section 3.2, for detailed description].

4.3 **STATISTICAL METHODS**

Data are presented as means and standard errors (SE) for SI by volume of haemorrhage. Repeated measures analysis of variance (ANOVA) was used to compare SI between the mild TBI group and the sham injury group, and the moderate TBI group and sham injury group. A test for interaction of group by volume of haemorrhage was used to determine if there were significant differences in the trend lines between acute TBI of mild and moderate severity compared to sham injury.

Pearson correlations were calculated for HR, SBP and SI by percentage volume of haemorrhage for each group individually. With the onset of blood volume loss ‘unloading’ of the arterial baroreflex results in a reduction in cardiac vagal tone and sympatheexcitation which acts to maintain MAP to near normal levels up to 20-30% blood volume loss (Evans, Ventura et al. 2001). That is up to Class 2 haemorrhage as classified by the ATLS (American College of Surgeons 2004). Beyond 30% blood volume loss the normal vital signs of BP and HR would generally have a good correlation with clinical shock. Separate correlations were therefore calculated for the subsets of up to 30% and 30-40% haemorrhage for each group.

The haemodynamic response to blood loss is significantly altered following acute TBI (McMahon 2007). Modification of the normal bi-phasic response to haemorrhage may alter the relationship between HR and BP with progressive blood loss. This could affect the performance of SI as an indicator of blood volume loss in the presence of
acute TBI. There was a marked difference in the bi-phasic haemodynamic response to haemorrhage in the moderate group between survivor and non-survivors. A further subgroup analysis was therefore undertaken using repeated measures ANOVA comparing the trend for SI with increasing volume of haemorrhage in survivors versus non-survivors. A test for interaction was used to examine whether the patterns of response were different between the two subgroups. The Univariate F-statistic was used with Geisser-Greenhouse adjustment. Significance levels at P<0.05 were assumed. JMP (version 5) and SAS software was used to analyze the data (SAS Institute Inc.).

4.4 RESULTS

Following sham injury the correlation of HR to percentage blood volume loss of less than 30% and 30 - 40% blood volume loss was poor (r = 0.3 and r = 0.4, respectively) (Table 7). SBP and SI correlated well with blood volume loss up to 40% (Table 7).

Analysis of the mild TBI group revealed the pattern of SI response to progressive loss of 40% blood volume between mild TBI and sham injury revealed no significant difference between the two groups (test of interaction p=0.39). (Figure 14). The correlation of HR, SBP in the mild injury group for haemorrhage of less than 30% and 30-40% was poor (Table 7). Following mild TBI, SI was also poorly correlated in both subsets of blood volume loss (r = 0.4 for both), (Table 7). There was no mortality in the mild TBI group during the observation period.

Comparison of the trend of SI response to haemorrhage between moderate TBI and sham injury using repeated measures analysis (MANOVA) revealed no significant
difference until 40% blood volume loss, when a significant difference in shock index between the two groups was identified (p=0.048), (Figure 14). The correlations of HR and SBP with blood volume loss of less than 30% was poor (r = 0.2), but a better association of SBP (r = -0.6) and SI (r = 0.6) with blood volume loss of less than 30% was observed (Table 7).

Four of the moderate TBI group died suddenly after the completion of haemorrhage, at time 45, 55, 60 and 90 minutes respectively. Further sub-group analysis of the moderate TBI group using a repeated measures analysis (MANOVA) revealed a significant difference in the trend lines of SI between the survivor subgroup compared to the non-survivor subgroup (p=0.0070), (Figure 15). SI failed to rise as expected with increasing haemorrhage in the non-survivor group.

4.5 DISCUSSION

Shock index is considered a more sensitive measure of increasing blood volume loss than the traditional vital signs in a wide range of clinical scenarios including gastrointestinal haemorrhage, ectopic pregnancy and haemorrhage secondary to trauma (Allgower and Burri 1968), (Little 1990), (Rady, Nightingale et al. 1992), (Birkhahn, Gaeta et al. 2003), (Birkhahn, Gaeta et al. 2005). Acute TBI alters the bi-phasic haemodynamic response to haemorrhage (McMahon 2007). Therefore the performance of SI may be altered when haemorrhage occurs in the presence of acute TBI. This investigation revealed that the use of SI in the presence of acute TBI could result in underestimation of the extent of underlying haemorrhage.
In keeping with previous reports we found that SI correlated well with haemorrhage in the absence of acute TBI (Allgower and Burri 1967), (Rady, Smithline et al. 1994), (Birkhahn, Gaeta et al. 2005). The correlation of SI with haemorrhage was reduced following acute TBI. In the moderate TBI, subgroup analysis of the survivors compared to non-survivors revealed a marked difference in the SI trend lines with increasing volume of haemorrhage. There was a marked dampening of the expected elevation of SI with haemorrhage in the non-survivor subgroup. SI appears to be a less reliable indicator of blood volume loss in the presence of acute TBI.

4.5.1 LIMITATIONS

This study did not permit identification of the mechanisms underlying the observed alteration in the diagnostic utility of SI in the presence of acute TBI. One possible explanation would be that there is a degree of decoupling of the relationship between HR and BP, resulting from a disturbance in central cardiovascular regulation following acute TBI. Autonomic uncoupling has been previously reported in patients with severe brain injury of differing aetiologies (Goldstein, Toweill et al. 1998).

Previous work considered a putative relationship between SI and cardiac function (Little 1990), (Rady, Nightingale et al. 1992). Little et al proposed that if SI is considered in the context of Ohm’s law (pressure = cardiac output x total peripheral resistance). SI becomes 1/ (stroke volume x total peripheral resistance) which approximates to the inverse function of cardiac work. This was confirmed by the analysis of the relationship between SI and left ventricular stroke work (LVSW) (Rady, Nightingale et al. 1992). SI is negatively related to LVSW. It is difficult to relate these
theories with the altered performance of SI in the presence of acute TBI. It is possible that the augmented catecholamine response to haemorrhage in the presence of acute TBI (Atkinson 2000) may have a role to play in altered LVSW; however this was not formally studied in this investigation.

4.5.2 CONCLUSIONS

SI has been shown to be more sensitive in the early identification blood volume loss in a number of previous clinical and laboratory investigations (Allgower and Burri 1967), (Little 1990), (Rady, Smithline et al. 1994), (Birkhahn, Gaeta et al. 2003), (Birkhahn, Gaeta et al. 2005). The principal finding of this investigation is that the relationship between SI and haemorrhage is altered following acute TBI. The use of SI in the assessment blood volume loss and response to resuscitation in patients with acute TBI as part of their injury should be interpreted with caution.
Figure 14. Mean Shock index (± SE) with increasing volume of haemorrhage following Mild TBI, Moderate TBI and Control (Sham) injury.
Figure 15. Mean Shock index (± SE) with increasing volume of haemorrhage in survivors versus non-survivors subgroups of moderate TBI.
Table 7. Correlations for HR, MBP and SI with volume haemorrhage (p values) in control (sham) injury, mild and moderate TBI.

<table>
<thead>
<tr>
<th>Group</th>
<th>HR</th>
<th>SBP</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control injury &lt;30% haemorrhage.</td>
<td>r = -0.34 (p=0.006)</td>
<td>r = -0.72 (p&lt;0.0001)</td>
<td>r = 0.66 (p&lt;0.0001)</td>
</tr>
<tr>
<td>Control injury &gt; 30% haemorrhage.</td>
<td>r = -0.40 (p=0.030)</td>
<td>r = -0.62 (p&lt;0.001)</td>
<td>r = 0.79 (p&lt;0.0001)</td>
</tr>
<tr>
<td>Mild TBI &lt; 30% haemorrhage.</td>
<td>r = 0.13 (p=0.316)</td>
<td>r = -0.44 (p&lt;0.001)</td>
<td>r = 0.43 (p=0.0004)</td>
</tr>
<tr>
<td>Mild TBI &gt;30% haemorrhage.</td>
<td>r = -0.59 (p&lt;0.001)</td>
<td>r = -0.55 (p=0.0013)</td>
<td>r = 0.38 (p=0.035)</td>
</tr>
<tr>
<td>Moderate TBI &lt;30% haemorrhage.</td>
<td>r = 0.16 (p=0.20)</td>
<td>r = -0.60 (p&lt;0.0001)</td>
<td>r = 0.56 (p&lt;0.0001)</td>
</tr>
<tr>
<td>Moderate TBI &gt;30% haemorrhage.</td>
<td>r = -0.28 (p=0.13)</td>
<td>r = -0.15 (p=0.43)</td>
<td>r = 0.88 (p&lt;0.0001)</td>
</tr>
</tbody>
</table>
CHAPTER 5

THE EFFECT OF ACUTE TRAUMATIC BRAIN INJURY OF MILD AND MODERATE SEVERITY ON THE ARTERIAL BAROREFLEX

5.1 INTRODUCTION

The integrity of the arterial baroreflex is central to cardiovascular homeostasis (Spyer 1984). It is one of the principal reflex mechanisms responsible for autonomic balance. Baroreflex responses triggered by haemorrhage are a life conserving measure aimed at maintaining perfusion of vital organs (Evans, Ventura et al. 2001). Acute TBI could be potentially complicated by acute disruption of the arterial baroreflex either as a result of direct neuronal injury or changes in neurotransmitter function complicating primary brain injury. Alteration in baroreflex function complicating acute TBI could create instability within the finely regulated closed-loop negative feedback system of the baroreflex (Cavalvanti 1996), (Hammer 2005).

The normal response to progressive simple haemorrhage is a neurally mediated bi-phasic HR and BP response (Barcroft, Edholm et al. 1944), (Evans, Ventura et al. 2001). Acute TBI of mild and moderate injury severity are associated with a significant modification of this bi-phasic haemodynamic response (McMahon 2007). This effect is graded according to the severity of induced injury. Following mild acute TBI the bi-phasic response is maintained but the depressor phase is attenuated. Moderate TBI was associated with a more marked attenuation of the depressor phase and a 50% mortality rate. In the non-survivor group the bi-phasic response was completely abolished with a
paradoxical effect of HR and BP maintained at near normal levels during significant haemorrhage in this group.

Soft tissue injury, which commonly complicates multi-system trauma, also results in an attenuation of the bi-phasic response to progressive simple haemorrhage (Overman 1947), (Little, Marshall et al. 1989). This is associated with an adverse effect on survival (Overman 1947). Furthermore, soft tissue injury is associated with a depression and rightward resetting of baroreflex function (Redfern, Little et al. 1984), (Jones 1989), (Anderson, Little et al. 1990).

Severe TBI is associated with significant autonomic dysfunction (Lowensohn, Weiss et al. 1977), (Winchell and Hoyt 1997), (Goldstein, Toweill et al. 1998). This dysfunction is proportional to the degree of neurological insult (Goldstein, Kempski et al. 1996). The effect of acute TBI on baroreflex function, an important determinant of autonomic balance, is unknown.

In this study the effects of acute TBI of mild and moderate severity on the baroreflex were investigated. The focus of this investigation was specifically on the effect(s) of less severe brain injury on cardiovascular reflex mechanisms, where critical changes in cardio regulatory control may be unsuspected clinically. Three groups of animals were studied; mild, moderate and sham (control) TBI. Baroreflex function was assessed using the phenylephrine pressor test adapted for the rat (Smyth, Sleight et al. 1969), (Jones, Kirkman et al. 1990). This experimental model enabled the effects of different severities of acute TBI on baroreflex function to be studied in the absence of common
clinical confounding variables such as alcohol, age and pre-morbid factors (Zink and Feustel 1995), (Zink, Maio et al. 1996), (Susman, DiRusso et al. 2002).

5.2 **STUDY AIMS:**

Evaluation of the effects of mild and moderate acute TBI on the arterial baroreflex.

5.3 **MATERIALS AND METHODS**

Male Wistar rats (Porton strain) weighing between 240-260g were used to study the effects of acute TBI, using the LFP injury model, on baroreflex function (Little and Redfern 1981), (Toulmond, Duval et al. 1993). The study animals were kept since weaning on a 12 hours light/dark cycle and fed on Beekay standard rat and mouse diet (B & K Universal, UK).

The present investigation was conducted with Home Office approval issued in conjunction with the Animal (Scientific Procedures) Act 1986, which conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (United States 1996).

5.3.1 **SURGICAL PREPARATION**

Anaesthesia was induced using isoflurane (Abbott Laboratories, UK; 3% in a 1:1 mixture of O₂/N₂O, FiO₂=0.5) in an anaesthetising chamber until cessation of spontaneous movement, loss of the righting reflex, absence of blink reflex and no
withdrawal to a noxious stimulus (pinch test applied to the foot). Surgical anaesthesia was maintained using 2-3% isoflurane administered via a mask. Both of the lateral tail veins and the ventral tail artery were cannulated using polyethylene cannulae (2FG and 3FG respectively, Portex Limited). Cannulae were pre-filled with heparinised saline (10IU. ml⁻¹ in 0.9% saline). The arterial line was utilized to measure arterial blood pressure using a strain gauge manometer (Sensonor 840, SensoNor, Norway). Electrocardiogram (ECG) needle electrodes were placed subcutaneously on the ventral surface of the animal to record the ECG, from which the heart rate was measured. A neonatal respiratory monitor was attached externally to the chest wall and connected to a transducer to monitor respiratory movements. Colonic temperature was monitored with a thermocouple inserted 6-8cm past the anal sphincter into the colon and was maintained at 37.8 ± 0.5°C (normal temperature for the rat) using a fan, blanket and overhead heating lamp.

On completion of the surgical procedure the isoflurane and N₂O were discontinued and anaesthesia was maintained using continuous intravenous infusion of alphaxalone/alphadalone (Saffan, Pitman-Moore, UK, 18-20mg. Kg⁻¹. h⁻¹ i.v.) using an infusion pump (Harvard 22, Harvard apparatus, UK). The anesthetised animals spontaneously breathed oxygen enriched air. The animal was then transferred to a stereotaxic frame.

5.3.2 LATERAL FLUID PERCUSSION MODEL

A craniotomy was performed stereotactically at the level of the right parietal cortex (3.5mm anterior to and 6mm above the interaural line leaving the dura intact. Teflon
tubing (2mm inner diameter) was placed directly over the dura and sealed in situ using methylmetacrylate cement. This was connected through a fluid filled system to an HPLC pump (SF400 Spectroflow, Kratos analytical instruments, NJ, USA). The HPLC pump was used to deliver a predetermined level of fluid percussion pressure, which was released onto the lateral cortex using an electronically controlled solenoid valve (RS, UK). Thus a brief pulse (20ms) of sterile water was applied to the brain at an applied cortical pressure of 1.2atm, producing mild injury and 1.8atm for moderate injury (Toulmond, Duval et al. 1993). Sham operated animals underwent identical procedures, but had no applied cortical pressure.

5.3.3 Phenylephrine pressor test

The baroreflex was assessed by determining the relationship between heart period (R-R interval) and systolic blood pressure (SBP) using the modification of the phenylephrine pressor test adapted for the rat (Smyth, Sleight et al. 1969), (Jones, Kirkman et al. 1990). Blood pressure was elevated using an increasing infusion of the α1-adrenergic receptor agonist, phenylephrine, over 4 steps between 8.3μl. min⁻¹ to 83μl. min⁻¹ over a period of 120 seconds, until SBP rose and reached a plateau level. The infusion was then discontinued and a resting period of at least 20 minutes was allowed before the test was repeated. An average of 41μg.kg⁻¹ of phenylephrine in 0.07ml of 0.9% normal saline was infused for each baroreflex assessment. A regression line was constructed using linear regression analysis by plotting heart period against SBP, the slope of the linear portion of this regression line was taken as an index of baroreflex sensitivity, while a lateral shift of the line indicated a resetting of the reflex (Smyth, Sleight et al. 1969).
A correlation coefficient was calculated for points lying in the linear mid-portion of the slope and its statistical significance assessed (Snedecor and Cochrane 1967), a value of P<0.05 being regarded as significant (Smyth, Sleight et al. 1969).

5.3.4 **Experimental Protocol**

This rate of infusion of alphadolone/alphaxolone was adjusted to allow a mild withdrawal and a 10mmHg rise in blood pressure in response to a pinch test applied to the Achilles tendon. This was appropriate after completion of induced TBI and was directed at avoiding excessive levels of anaesthetic agent which would have resulted in depression of the arterial baroreflex sensitivity (Sear and Prys-Roberts 1979). A minimum of 60 minutes was allowed to elapse before physiological data were recorded to allow an adequate washout period of isoflurane before commencement of the experimental protocol. The timelines of the experimental protocol are reproduced in (Figure 16). Experiments were performed on three groups of six animals. Animals were randomly allocated to the mild TBI, moderate TBI or control (sham) TBI group. During the washout period of isoflurane in preparation for baroreflex assessment the phenylephrine cannula was primed with phenylephrine (150μg. ml⁻¹) by infusion at 146μl. min⁻¹, until an initial rise in blood pressure was seen. The animal was allowed to recover for a minimum of 20 minutes before the control baroreflex sensitivity test was undertaken. Each animal acted as its own control. A further 20 minutes was allowed to elapse before LFP (sham) injury was induced. The LFP injury was associated with a brief apnoeic response and a transient rise in MAP. Equilibration of baseline physiological variables following the induced TBI was facilitated by allowing 10
minutes to elapse. Baroreflex sensitivity was assessed 10 minutes and 30 minutes after the induced or sham TBI.

Physiological measurements were recorded using a computerized physiological data acquisition system (Fastdaq, Lectromed, UK. Real time data capture, display and analysis system), (Table 8). Blood gas analyses were performed before the control baroreflex sensitivity test and 10 minutes after induced brain injury (ABL330, Radiometer, Denmark), (Table 9).

Animals were killed humanely using a lethal injection of Sagital anaesthesia at the end of the experiment. Post-mortem examination of the brain was carried out on all animals immediately following the procedure.

5.3.5 **Statistical Methods**

Values are presented as means ± SEM, unless otherwise indicated. The relationship between heart period (R-R interval) and SBP at baseline (Tcon), before TBI, 10 minutes (T10) and 30 minutes (T30) post-injury, was examined for each group (moderate, mild and control) using linear regression for individual animals. Each animal acted as its own control. The regression coefficient (slope of the regression line or \( \beta \)) was taken as an index of baroreflex sensitivity. Repeated measures ANOVA was used to examine the trend in baroreflex before (Tcon) and after TBI (T10 and T30) within and between the control and injury groups. The Greenhouse-Geisser adjustment was used. Analysis was limited to animals with a correlation coefficient for the linear portions of the slopes above 0.8, which were significantly different from zero (Ponikowski, Chua et al. 2001),
(Hunt and Farquhar 2005). Analysis was performed using JMP (SAS Institute Inc, version 5). Significance at \( p < 0.05 \) was assumed.

This study was powered at greater than 90\% with \( n=6 \) animals per group to show a statistically significant difference (2-sided \( p=0.05 \)) of an effect of 3 or more (difference in weighted mean of 0.09 (SD=0.03) in baroreflex sensitivity between pre and post TBI in the moderate group (Jones, Kirkman et al. 1990).

5.4 RESULTS

5.4.1 BASELINE RESULTS

Baseline physiological parameters of body weight, HR, MAP, arterial blood gases, respiratory rate and core body temperature were assessed prior to the control baroreflex sensitivity test. There was no significant difference in baseline physiological parameters observed between the three study groups (Table 8, Table 9).

Acute TBI resulted in a brief apnoeic period. Significant differences in the duration of apnoea were observed according to the severity of induced injury. No apnoea occurred in the control (sham) injury group. Mild TBI was associated with an apnoeic period of 4.17 ± 1.0 sec compared to 10.2 ± 1.6 sec (\( P< 0.001 \)) in moderate TBI (Table 8).

Acute TBI was also associated with a transient rise in blood pressure (pressor response). Following moderate TBI there was pressor response of 26.3 ± 9.3mmHg compared to 6.33 ± 7.4mmHg following mild TBI. There was a significant difference in the pressor
responses between the two groups (p=0.002). No pressor response was observed in the control (sham) injury group (Table 8).

Physiological parameters that can influence the baroreflex sensitivity including HR, MAP, respiratory rate and PaO₂ were not significantly different between groups when compared at control, T10 and T30 (Table 9). No hypoxic episodes were observed in any of the study animals (Table 8). However there was a reduction in PaO₂ observed in the mild group between the pre- and post-TBI time intervals (214.3 ± 85.4mmHg; 152.5 ± 41.0mmHg), (Table 9). This was not associated with any significant change in PaCO₂. In the moderate TBI group PaCO₂ increased from 36.9 ± 4.4mmHg pre-TBI to 47.6 ± 4.0mmHg in the post-TBI phase. Core body temperature was maintained in the normal physiological range throughout the study period.

5.4.2 Baroreflex Sensitivity

The correlation co-efficient for the linear portions of the slopes in all of the study groups were significantly different from zero (r > 0.8) with the exception of one animal in the control group which was therefore subsequently omitted from the final analysis.

Control values of baroreflex sensitivities were recorded prior to the induced (or sham) TBI. Repeated measures ANOVA revealed no significant association of baroreflex over time (p=0.21).
In the mild TBI group there was a trend towards an increase in sensitivity observed, with a relative increase in baroreflex sensitivity of 45% and 54% at 10 and 30 minutes post TBI respectively (Figure 19). This did not reach statistical significance (p = 0.152).

In the moderate TBI group there was a relative increase in baroreflex sensitivity of 125% and 101% at 10 and 30 minutes post TBI respectively (Figure 19). Analysis of the moderate TBI group using repeated measure ANOVA revealed significant differences in baroreflex sensitivity at T10 and T30 (F-ratio=10.18, p=0.005) compared to pre-TBI values. Repeated measures ANOVA comparing the trends between the three groups indicated significant differences between the control and moderate TBI only (F ratio=6.26, p=0.01). An example of the effect of acute TBI of moderate severity on baroreflex sensitivity is illustrated in Figure 17. An example of baroreflex sensitivity following sham injury is illustrated in Figure 18. Similar trends in baroreflex sensitivities were observed in all animals studied in the moderate group (Figure 20; Table 10).

5.4.3 POST MORTEM EXAMINATION

Post mortem evaluation of the moderate TBI group revealed macroscopic evidence of contusion on the surface of the ipsilateral cortex with small amounts of subarachnoid hemorrhage as described in other reports (Thompson, Lifshitz et al. 2005). The mild and control animals had no macroscopic evidence of brain injury.
5.5 **DISCUSSION**

Over the past 25 years there has been a significant improvement in the outcome from isolated TBI (Patel, Bouamra et al. 2005). Mortality following severe TBI has been reduced by approximately 50% with similar improvements in functional outcome. This is predominantly the result of a substantial improvement in the early management of multiply injured patients and developments in neurointensive critical care (Sarrafzadeh, Peltonen et al. 2001), (Zink 2001). Improved understanding of the pathophysiological effects of acute TBI is likely to be important factor under-pinning further advances in therapeutic options for this patient cohort. This investigation reveals a significant alteration of baroreflex sensitivity following moderate TBI. A similar trend was observed following mild TBI. This observation has not been previously reported.

5.5.1 **THE EXPERIMENTAL MODEL**

Undertaking the investigation of the effects of acute TBI on baroreflex sensitivity in the pre-clinical setting avoided common confounding factors such as alcohol, medications, pre-morbid illness which complicate human studies in this area. These factors are known to modify baroreflex function and could confound the interpretation of the effects of acute TBI on baroreflex function in the clinical setting (Anderson, Little et al. 1990), (Zink and Feustel 1995), (Zink, Schultz et al. 1999). There is close species correlation in the cardiovascular responses to both haemorrhage and injury between man and rodents (Barcroft, Edholm et al. 1944), (Oberg 1970), (Secher, Sander Jensen et al. 1984), (Little, Randall et al. 1984), (Secher and Bie 1985).
The phenylephrine pressor test is a well validated quantitative test of baroreflex function both in man and animal studies (Smyth, Sleight et al. 1969), (Jones, Kirkman et al. 1990), (Colombo 1999). This technique, also termed the Oxford technique, was first developed by Smyth and his colleagues (Smyth, Sleight et al. 1969). It is a ‘gold standard’ technique for estimating cardiovagal baroreflex gain. Phenylephrine does not alter cerebral perfusion in the presence of brain injury which was an important consideration in this study (Johnston, DeWitt et al. 1994). We did not assess the baroreflex response to lowered blood pressure, using pharmacological agents such as sodium nitroprusside, because nitrovasodilators have recently been shown to have significant extra-vascular effects which confounds the interpretation of baroreflex function (Casadei and Paterson 2000). Furthermore, this would probably have resulted in additional secondary brain injury in the injury groups which would have added further systematic bias into comparisons between sham injury and brain injury groups (Chesnut 1997), (Jeremitsky, Omert et al. 2003), (Trabold, Schueler et al. 2006).

The choice of anaesthetic agent used in this model was critically important. Many anaesthetic agents modify baroreflex function and the injury-induced modulations of baroreflex activity. Intravenous alphadolone/alphaxalone anaesthesia preserves baroreflex function and the effects of injury-induced modulations of the arterial baroreflex (Timms 1981), (Faber 1989), (Mackway-Jones, Foex et al. 1999). The dose of anaesthesia was carefully adjusted to maintain adequate anaesthesia throughout the experimental period, avoiding excessive doses which would have resulted in a dose-dependent reduction in arterial pressure (Sear and Prys-Roberts 1979).
The LFP model of brain injury is one of the most widely used experimental models of brain injury because of its construct validity with closed head injury in man (Dixon, Clifton et al. 1991), (Toulmond, Duval et al. 1993), (Laurer and McIntosh 1999). This model allowed graded and reproducible severities of brain injury to be induced. The level of injury up to and including moderate TBI is associated with no mortality and no major neurological deficit (Toulmond, Duval et al. 1993). The histological changes resulting from this model include a well defined ipsilateral cortical injury, ipsilateral hippocampal and amygdaloid injury (Toulmond, Duval et al. 1993), (Thompson, Lifshitz et al. 2005). Other aspects of molecular, vascular and behavioural correlates of different severities of human brain injury are also closely replicated in this model (Toulmond, Duval et al. 1993).

5.5.2 Principal Findings

Acute TBI of moderate severity was associated with a significant increase in baroreflex sensitivity. The average increase in baroreflex sensitivity at 10 and 30 minutes after acute moderate TBI was 125% and 101% respectively. A similar trend was observed following mild TBI, where there was an average increase in baroreflex sensitivity of 45% and 54% respectively. The increase in baroreflex sensitivity was closely related to the severity of induced TBI in the mild to moderate range. Following moderate TBI there was a trend towards a leftward re-setting (towards a bradycardia) of the baroreflex. However, this occurred in the presence of a significant change in the baroreflex slope (sensitivity), therefore quantification of the degree of re-setting could not be accurately assessed.
There was no significant difference in the pre-test values of respiratory rate, HR or MAP either within or between the groups at the time baroreflex tests were conducted. This was an important consideration, as significant changes in these physiological values could also contribute to alterations in baroreflex sensitivity (Kirchheim 1976). A significant increase in PaCO$_2$ was observed following moderate TBI. Hypercapnia results in an increase in MAP due to an activation of sympathetic nervous system (via central chemoreceptors) and a decrease in HR due to a secondary reflex activation of the parasympathetic nervous system (via arterial baroreceptors) in response to the rise in MAP (Oikawa, Hirakawa et al. 2005). Hypercapnia, however, does not have a direct effect on baroreflex sensitivity (Oikawa, Hirakawa et al. 2005).

Animals breathed oxygen enriched air in order to avoid hypoxic episodes occurring immediately after the induced brain injury, as hypoxia is associated with a reduction in baroreflex sensitivity (Kongo, Yamamoto et al. 1999), (Atkinson 2000). Consequently PaO$_2$ levels were higher than normal in all groups. Acute administration of oxygen increases the baroreflex sensitivity (Waring, Thomson et al. 2003), however this was controlled for within this experimental design. A drop in arterial oxygen content could result in a reduction in arterial baroreflex sensitivity. In the mild TBI group, a fall in the mean PaO$_2$ occurred following TBI from 214mmHg to 151mmHg. This may have resulted in an underestimation of the effect of mild TBI on baroreflex sensitivity.

The pressor response to acute TBI is thought to result from a transient catecholamine surge occurring immediately after TBI (Atkinson 2000). A pressor response also occurs following major tissue damage (Redfern, Little et al. 1984). The effects of soft tissue injury, however, on baroreflex function are the opposite of those observed following
acute TBI. Musculoskeletal injury is associated with a reduction in baroreflex sensitivity and a rightward re-setting of the baroreflex (Redfem, Little et al. 1984), (Anderson, Little et al. 1990). It appears therefore, that the cardiovascular pathophysiology of acute TBI differs to that of musculoskeletal injury. Acute TBI appears to result in a combination of increased sympathetic activity (pressor response) and increased vagal activity.

An increase in baroreflex sensitivity immediately following acute TBI could be advantageous, with an increased HR response protecting against a fall in blood pressure in the presence of acute neuronal injury. This sharp rise in baroreflex sensitivity may however be detrimental, as it could result in instability within the closed loop negative-feedback system of the baroreflex (Spyer 1984), (Pilowsky 2002). The principal afferent nerves of the baroreflex are a mixture of myelinated and non-myelinated nerve fibres resulting in different speeds of nervous impulse transfer (Borst and Karemaker 1983). Control systems that contain time delays and exhibit powerful responses can become unstable. Therefore there is potential for instability following sudden shifts in the baroreflex sensitivity/gain due to the time lag differences of neuronal transmission within this ‘closed loop system’ (Keyl, Schneider et al. 2001), (Hammer 2005). This would be expected to be manifested clinically as instability in blood pressure control. Evidence suggests that patients with TBI are prone to frequent episodes of hypotension despite targeted therapy to prevent hypotension (Jones, Andrews et al. 1994), (Jeremitsky, Omert et al. 2003).
5.5.3 LIMITATIONS OF THE STUDY

This investigation was confined to the short term assessment of the cardiовagal limb of the baroreflex. There is evidence that both limbs of the baroreflex can be affected differently. There are important changes in baroreflex function identified in this investigation. Further assessment of the autonomic system using longer term models incorporating the assessment of the cardiовagal and sympathetic limbs of the response would be important. It would also be important to correlate changes in autonomic function with the loci of central nervous system injury.

5.5.4 CONCLUSIONS

Acute TBI of moderate severity was associated with a significant increase in baroreflex sensitivity. This increase in baroreflex sensitivity was closely related with the severity of induced TBI and was observed in both mild and moderate grades of acute TBI. This increase in baroreflex sensitivity may be a protective mechanism minimising reductions in cerebral perfusion in the acute post injury period. This sudden marked increase in baroreflex sensitivity after moderate TBI could however result in instability within the closed-loop control system of the arterial baroreflex. It represents a significant modification of the normal cardiovascular homeostatic mechanisms. Evaluation of baroreflex function following acute TBI in the clinical setting would be important to further our understanding of the pathophysiological impact of acute TBI on cardiovascular regulation.
Figure 16. Schematic diagram to indicate the timeline of the study protocol.

-20  Control Baroreflex Assessment

T 0  Lateral Fluid Percussion TBI

T 10  Baroreflex Assessment

T 30  Baroreflex Assessment
The relationship between heart period (HP) and systolic pressure (SBP), 10 minutes and 30 minutes after acute TBI of moderate severity using the phenylephrine pressor test in one animal anesthetised with alphadalone/alphaxalone.
The relationship between heart period (HP) and systolic pressure (SBP), 10 minutes and 30 minutes after control (sham) TBI using the phenylephrine pressor test in one animal anesthetised with alphadalone/alphaxalone.
Figure 19. The relative changes expressed as a percentage, in the baroreflex sensitivity at 10 minutes, and 30 minutes following moderate, mild and control (sham) TBI.
Table 8. Baseline Physiological variables.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Mild (n=6)</th>
<th>Moderate (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>253.0 ± 8.9</td>
<td>252.8 ± 9.2</td>
<td>259.0 ± 9.5</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>37.85 ± 0.03</td>
<td>37.83 ± 0.04</td>
<td>37.83 ± 0.06</td>
</tr>
<tr>
<td>Hb</td>
<td>11.47 ± 0.56</td>
<td>11.37 ± 1.04</td>
<td>11.52 ± 0.45</td>
</tr>
<tr>
<td>pH</td>
<td>7.33 ± 0.00</td>
<td>7.34 ± 0.03</td>
<td>7.34 ± 0.02</td>
</tr>
<tr>
<td>ABE (mmol/l-1)</td>
<td>-3.62 ± 2.10</td>
<td>-2.72 ± 2.99</td>
<td>-0.54 ± 2.92</td>
</tr>
<tr>
<td>BicarB</td>
<td>20.92 ± 2.84</td>
<td>22.5 ± 1.52</td>
<td>25 ± 2.82</td>
</tr>
<tr>
<td>PaO₂</td>
<td>192.2 ± 21.0</td>
<td>152.5 ± 41.0</td>
<td>218.6 ± 67.7</td>
</tr>
<tr>
<td>PaCO₂*</td>
<td>37.2 ± 6.5</td>
<td>40.4 ± 4.4</td>
<td>47.6 ± 4.0*</td>
</tr>
<tr>
<td>Apnoea Period (sec)*</td>
<td>0.0 ± 0.0</td>
<td>4.17 ± 1.02</td>
<td>10.16 ± 1.75*</td>
</tr>
<tr>
<td>Pressor response (mmHg)*</td>
<td>0.0 ± 0.0</td>
<td>6.33 ± 7.4</td>
<td>26.3 ± 9.3*</td>
</tr>
</tbody>
</table>

Number of rats (n): heart rate (HR): mean arterial pressure (MAP): arterial oxygen pressure (PaO₂): carbon dioxide tension (PaCO₂).

Values are expressed as Mean ± SD, *p < 0.05 ANOVA comparison pre/post values between all three groups.
Table 9. Physiological variables pre- and post- TBI in sham, mild and moderate TBI groups.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Mild (n=6)</th>
<th>Moderate (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-TBI</td>
<td>Post-TBI</td>
<td>Pre-TBI</td>
</tr>
<tr>
<td>HP</td>
<td>130.7 ± 5.1</td>
<td>135.0 ± 6.2</td>
<td>127.9 ± 13.8</td>
</tr>
<tr>
<td>MAP</td>
<td>120.5 ± 8.0</td>
<td>114.5 ± 13.2</td>
<td>117.7 ± 13.2</td>
</tr>
<tr>
<td>PaO₂</td>
<td>193.6 ± 31.6</td>
<td>192.2 ± 21.0</td>
<td>214.3 ± 85.4</td>
</tr>
<tr>
<td>PaCO₂*</td>
<td>38.3 ± 3.2</td>
<td>37.2 ± 6.5</td>
<td>37.5 ± 4.6</td>
</tr>
<tr>
<td>Resp. rate</td>
<td>89.5 ± 7.0</td>
<td>91.0 ± 10.2</td>
<td>89.0 ± 9.4</td>
</tr>
</tbody>
</table>

Physiological variables pre- and post-acute TBI in control (sham), mild and moderate TBI groups. Number of rats (n): heart period (HP): mean arterial pressure (MAP): arterial oxygen pressure (PaO₂): carbon dioxide tension (PaCO₂): respiratory rate (resp. rate). Values are expressed as Mean ± SD. *p < 0.05 ANOVA comparison pre/post values between all three groups.
Table 10. The effects of control, mild and moderate acute TBI on baroreflex sensitivity (ms.mmHg⁻¹) expressed as means ± SD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Animal</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Weighted mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td></td>
<td>0.26 ± 0.01</td>
<td>0.51 ± 0.01</td>
<td>0.15 ± 0.01*</td>
<td>0.52 ± 0.01</td>
<td>1.08 ± 0.03</td>
<td>0.92 ± 0.01</td>
<td>0.45 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>T10min</td>
<td></td>
<td>0.19 ± 0.01</td>
<td>0.60 ± 0.01</td>
<td>0.09 ± 0.01*</td>
<td>0.62 ± 0.01</td>
<td>1.24 ± 0.01</td>
<td>1.03 ± 0.03</td>
<td>0.50 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>T30min</td>
<td></td>
<td>0.16 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>0.14 ± 0.01*</td>
<td>0.45 ± 0.01</td>
<td>1.27 ± 0.02</td>
<td>0.98 ± 0.01</td>
<td>0.51 ± 0.005</td>
</tr>
<tr>
<td>Mild</td>
<td>Control</td>
<td></td>
<td>0.30 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>1.50 ± 0.06</td>
<td>0.40 ± 0.02</td>
<td>0.24 ± 0.01</td>
<td>0.87 ± 0.01</td>
<td>0.49 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>T10min</td>
<td></td>
<td>0.55 ± 0.01</td>
<td>0.97 ± 0.04</td>
<td>1.30 ± 0.12</td>
<td>0.57 ± 0.06</td>
<td>0.34 ± 0.01</td>
<td>1.66 ± 0.03</td>
<td>0.59 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>T30min</td>
<td></td>
<td>0.49 ± 0.01</td>
<td>0.68 ± 0.01</td>
<td>1.65 ± 0.13</td>
<td>0.81 ± 0.04</td>
<td>0.33 ± 0.02</td>
<td>1.96 ± 0.08</td>
<td>0.59 ± 0.005</td>
</tr>
<tr>
<td>Moderate</td>
<td>Control</td>
<td></td>
<td>0.64 ± 0.02</td>
<td>0.46 ± 0.02</td>
<td>0.25 ± 0.01</td>
<td>0.35 ± 0.02</td>
<td>0.48 ± 0.01</td>
<td>0.37 ± 0.01</td>
<td>0.39 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>T10min</td>
<td></td>
<td>1.05 ± 0.02</td>
<td>0.98 ± 0.03</td>
<td>0.67 ± 0.02</td>
<td>0.51 ± 0.02</td>
<td>1.64 ± 0.04</td>
<td>0.82 ± 0.02</td>
<td>0.85 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>T30min</td>
<td></td>
<td>0.87 ± 0.02</td>
<td>1.27 ± 0.07</td>
<td>0.39 ± 0.02</td>
<td>0.74 ± 0.02</td>
<td>1.17 ± 0.02</td>
<td>0.67 ± 0.02</td>
<td>0.81 ± 0.01</td>
</tr>
</tbody>
</table>

(*Correlation co-efficient = < 8, therefore excluded from final analysis of data.)
Figure 20. Effect of control, mild and moderate acute TBI on baroreflex sensitivity (ms.mmHg\(^{-1}\)) expressed as means ± SD.
CHAPTER 6

FINAL DISCUSSION

6.1 INTRODUCTION

Traumatic brain injury is the leading cause of death and disability in young people (Murray, Teasdale et al. 1999), (Thurman, Alverson et al. 1999), (Zink 2001). Advances in systems of trauma care have been associated with improved survival following both multiple trauma and isolated brain injury (Shackford, Mackersie et al. 1987), (Lecky, Woodford et al. 2000). This improvement in survival is markedly reduced when acute TBI is a component of multiple trauma (Patel, Bouamra et al. 2005). Severe TBI is associated with a high mortality and morbidity. The focus of this research was on mild to moderate TBI where an adverse effect on mortality and the integrity of cardiovascular homeostasis may be unexpected.

6.1.1 ANALYSIS OF THE UK TARN DATABASE

Analysis of the UK TARN database was conducted in a study population limited to patients who had sustained blunt trauma with an ISS between 16 and 50. These parameters were chosen to reflect a range of injury severity where TBI could be expected to affect mortality. The TBI group had sustained a single AIS 3 brain injury combined with extracranial injury, contributing to their overall injury severity. The control group consisted of patients with extracranial injury only. The probability of death was modelled using logistic regression adjusting for injury severity and age. The
mortality of an isolated AIS 3 brain injury was < 4.7%. This did not differ significantly from the mortality of isolated AIS 3 injuries in the other main body regions. Adjusting for injury severity mortality from multiple trauma was doubled in the presence of moderate TBI (odds ratio 2.08, 95% CI, 1.57-2.77), (McMahon, Yates et al. 1999). This observation led to the hypothesis that acute TBI could modify cardiovascular homeostasis, and thereby contribute to the additional mortality observed when TBI and extracranial injuries are combined.

Systematic review of the literature revealed that there was a dearth of research evaluating the effects of acute TBI on cardiovascular homeostasis. Clinically-based research in this area is complicated by the number of confounding factors that render interpretation of cardiovascular responses difficult. Factors common to the clinical setting include: the heterogeneous distribution and severity of injuries, age, pre-morbid factors, alcohol, anaesthetic and opioids which can interact with TBI to alter cardiovascular homeostatic responses (Schadt and Ludbrook 1991), (Ohnishi, Kirkman et al. 1997), (Mihlham and LaMorte 2004), (Demetriades, Kuncir et al. 2006), (Flaada, Leibson et al. 2007). To avoid these confounding variables investigation into the effects of TBI on the bi-phasic haemodynamic response to haemorrhage and into the effects of TBI on the arterial baroreflex were undertaken in a laboratory setting.
6.1.2 **The effect of acute TBI on the bi-phasic haemodynamic response to haemorrhage**

Evaluation of the effect of acute TBI on the cardiovascular response to haemorrhage is fundamental to our understanding of the pathophysiology of acute TBI and cardiovascular homeostasis. Systematic review of the literature identified some laboratory evidence of impaired cardiovascular compensation for haemorrhage following acute TBI (Yuan and Wade 1991), (Yuan and Wade 1992), (Law, Hovda et al. 1996). No published work was identified that examined the effects of acute TBI on the bi-phasic haemodynamic response to haemorrhage.

This investigation revealed that both mild and moderate TBI significantly altered the bi-phasic HR and MAP response to progressive simple haemorrhage (McMahon 2007). This effect was graded according to the severity of induced injury. In the mild TBI group, phase 2 of the response was maintained, but was delayed in onset. Following moderate TBI phase 2 was significantly attenuated, resulting in the HR and MAP being maintained higher for longer during progressive haemorrhage. Fifty percent of this group died suddenly shortly after the completion of haemorrhage. Subgroup analysis of the moderate TBI group comparing survivors and non-survivors revealed that in non-survivors, phase 2 of the bi-phasic response was completely abolished. This acute modification of the bi-phasic haemodynamic response to haemorrhage may adversely affect compensation for haemorrhage and thereby contribute to the increased mortality seen when acute TBI and extracranial injuries are combined.

Alteration of the bi-phasic haemodynamic response to blood loss also results from soft tissue injury (Little, Jones et al. 1988), (Mackway-Jones, Foex et al. 1999), (Foex,
Kirkman et al. 2004). Experimental evidence gathered over 70 years ago demonstrated that sciatic nerve stimulation, as a model of soft tissue injury, increased mortality from 25 to 85% after haemorrhage (Overman 1947). Morphine, which mimics the effects of "injury" on the bi-phasic haemodynamic response also increases mortality after haemorrhage in the rat (Ohnishi, Kirkman et al. 1997).

The precise aetiology of the increased mortality observed when the bi-phasic response to haemorrhage is attenuated remains unclear. A number of theories have been proposed over the years but conclusive evidence in support of a the precise pathophysiology underlying this increased mortality remains elusive (Kirkman, Zhang et al. 1995), (Foex, Kirkman et al. 2004).

6.1.3 CHEMORECEPTOR FUNCTION

Analysis of arterial blood gases over the course of 40% haemorrhage revealed a normal pattern of PaCO₂ reduction with progressive haemorrhage in mild and moderate TBI. Whilst this was not a detailed assessment of the chemoreceptor function, which would have required assessment of tidal volumes and respiratory patterns, it suggests that the integrity of the chemoreceptor response was maintained even when the bi-phasic response to haemorrhage was attenuated or abolished.
6.1.4 **Shock Index**

Under normal conditions Shock Index (HR/SBP) has a linear relationship with percentage blood loss (Allgower M. 1967). This relationship is maintained when haemorrhage is associated with soft tissue injury (even when the bi-phasic response is altered) (Little 1990). SI has an advantage in that it is a more sensitive early indicator of haemorrhage than traditional vital signs (Allgower M. 1967), (Little 1990), (Rady, Nightingale et al. 1992), (Birkhahn, Gaeta et al. 2005). The ability of SI to reflect the extent of ongoing haemorrhage was compromised by the presence of TBI. There was marked flattening of the expected trend of increasing SI with progressive haemorrhage particularly in the non-survivor group of moderate TBI. This investigation suggests that SI is an unreliable early indicator of underlying haemorrhage in patients with a concomitant TBI.

6.1.5 **The effect of acute TBI on the arterial baroreflex**

The principal reflex mechanism responsible for the maintenance of arterial pressure is the arterial baroreflex (Spyer 1984), (Dampney 1994), (Pilowsky 2002). The effect of acute TBI on the arterial baroreflex has not been previously reported on.

Assessment of the effects of acute TBI of mild and moderate severity on the arterial baroreflex was undertaken using the phenylephrine pressor test adapted for the rat. This revealed that acute TBI was associated with an early increase in baroreflex sensitivity. This increased sensitivity was particularly pronounced in the moderate TBI group. An increase in baroreflex sensitivity is generally considered to be protective in the presence
of haemorrhage. This may not be the case however, as the arterial baroreflex operates within a finely regulated closed-loop negative feedback system. Closed loop negative feedback systems with integral components which have time lags built into the system are known to be associated with instability if there is a sudden shift in one of the key components of the feedback system (Keyl, Schneider et al. 2001), (Hammer 2005). Instability of the baroreflex system would be expected to be manifested clinically in instability in blood pressure control. Our experiment was conducted over a relatively short time frame and therefore BP instability could not be reasonably assessed in this particular set of tests, although this was observed following moderate TBI in the haemorrhage set of experiments (McMahon 2007), and has also been reported on in a clinical setting (Jones, Andrews et al. 1994), (Jeremitsky, Omert et al. 2003). In addition Law et al observed that when acute TBI was combined with haemorrhage this was associated with a loss of vasomotor tone and a poor blood pressure response to resuscitation (Law, Hovda et al. 1996).
6.2 TRANSLATION OF PRE-CLINICAL INVESTIGATIONS

An important consideration regarding the observations made in the laboratory studies undertaken in this research is how relevant they are to clinical practice. This research was largely undertaken in a laboratory setting, in order to control the size of brain injury and to avoid confounding factors commonly present in the clinical setting. Pre-clinical models of neurotrauma have greatly advanced our understanding of the pathophysiology of brain injury over the past two decades (McIntosh, Saatman et al. 1998), (Kazanis 2005). However there are recognised limitations of laboratory models of injury and neurotrauma (Statler, Jenkins et al. 2001). Therefore the principal objective of this pre-clinical study is a ‘proof of concept’ of the hypotheses under investigation, which will require further validation in the clinical setting.

A number of prerequisites were required for the optimum design of the models utilised in this work. In experimental terms the term ‘model’ is defined as a ‘simplified representation of a phenomenon’ (Merriam-Webster 2005). The experimental designs adopted in this work were chosen to optimally model the phenomenon of ‘closed head injury’ allowing investigation into the effects of acute TBI on the bi-phasic response to haemorrhage and on the arterial baroreflex to be studied in the absence of confounding variables.
6.2.1 Head Injury Model

The head injury model selected for this study was critically important. A pre-clinical model designed to model the clinical sequelae of closed head injury should fulfil a number of criteria:

- It should closely replicate the injury biomechanics of closed head injury in man.
- The inflicted injury should closely replicate the physiological, biochemical and behavioural components of human closed head injury.
- It should be possible to precisely vary the severity of injury, which should be reproducible and quantifiable between different investigators and laboratories.
- The damage should be a continuum, increasing in severity as the mechanical force is increased.

Human TBI is generally the result of a blunt injury which commonly results in a combination of focal and diffuse injuries. Focal brain injury is characterised by surface contusions, which may or may not be accompanied by skull fracture or haematoma formation. This type of damage is usually more pronounced at the site of impact and may also be associated with contra coup injury and varying degrees of white matter injury (diffuse axonal injury) (Laurer and McIntosh 1999), (Graham, McIntosh et al. 2000). The injury impact biomechanics of the LFP (<20msec) model closely match those of human concussive brain injury (Lindgren and Rinder 1966).

The histopathological changes associated with the LFP model include areas of discrete ipsilateral cortical injury, axonal damage, subarachnoid haemorrhage, tissue tears followed by focal necrosis and cell loss (McIntosh, Vink et al. 1989), (Toulmond,
Duval et al. 1993). Although the impact is unilateral it produces bilateral neuronal damage and diffuse white matter damage remote from the injury site resembling human white matter injury (Yaghmai and Povlishock 1992), (Saatman, Graham et al. 1998) (Graham, Raghupathi et al. 2000).

The immediate physiological responses to LFP injury comprises of a brief loss of consciousness, changes in blood pressure (pressor response), a brief apnoea, elevated ICP, decreased CPP and altered CBF (Dixon, Lighthall et al. 1988), (Pfenninger, Reith et al. 1989). Alterations in the CBF and increased permeability of the BBB following TBI in this model are similar to that seen in human TBI (McIntosh, Vink et al. 1989), (Schmidt and Grady 1993). CBF increases in the injured cortex to over 100% of baseline (Muir, Boerschel et al. 1992), and then falls to 40-50% of baseline within 15-30 minutes after injury (Yuan, Prough et al. 1988), (Muir, Boerschel et al. 1992); this lasts many hours after injury and is associated with uncoupling of cerebral glucose metabolism (Hovda. Lee et al. 1995). The altered ionic homeostasis, blood flow and biochemical changes seen in human TBI is also replicated in this model of TBI (Jaggi, Obrist et al. 1990), (Okiyama, Smith et al. 1992), (Bergsneider, Hovda et al. 1997).

From the physiological, biochemical, histological and molecular standpoint the LFP model shows a direct relationship between the majority of pathological alterations and injury severity of human closed head injury. Consequently it is the most widely accepted in vivo model for mechanistic studies on human TBI. It has the additional advantage that extensive normative data are available for this model (Povlishock, Hayes et al. 1994), (Thompson, Lifshitz et al. 2005).
Whilst animal models closely replicate closed head injury in man none of these models can fully reproduce human TBI (Laurer and McIntosh 1999), (Statler, Jenkins et al. 2001), (Kazanis 2005). The reason is not simply metaphysical; it lies in the fact that human cases of TBI are not themselves a uniform entity. In addition, the clinical scenario of acute TBI is often more complex with variables such as age, gender, co-existing extracranial injuries of differing severities and distribution, intoxication with alcohol or 'recreational' drugs, and pre-morbid illness all contributing to differing outcomes (Flamm, Demopoulos et al. 1977), (Teasdale, Skene et al. 1979), (Green, Cross et al. 1995), (Zink, Maio et al. 1996), (Susman, DiRusso et al. 2002), (Kuhne, Ruchholtz et al. 2005). There is a need however, to elucidate basic mechanisms underlying central nervous system pathophysiology using 'pre-clinical models', in order to inform the direction of future research in more complex models and in the clinical setting.
6.2.2 Choice of Anaesthesia

It would be unethical to conduct this work in conscious animals. The choice of anaesthetic agent used in the investigation of the effects of acute TBI on the bi-phasic response to haemorrhage and baroreflex activity was critically important. This is exemplified by the inconsistencies in the published work on cardiovascular physiology and haemorrhage. The original work on the cardiovascular responses to haemorrhage date back to World War II when Barcroft first described the bi-phasic response to progressive simple haemorrhage in human conscious volunteers (Barcroft, Edholm et al. 1944), (Warren 1945). Many textbooks of physiology, including the widely referenced Advanced Trauma Life Support Manual depict the cardiovascular responses to blood loss as a monophasic HR and BP response, with a progressive increase in sympatho-excitation as haemorrhage progresses (Secher and Bie 1985), (American College of Surgeons 2004). This is largely based on the experimental work conducted subsequent to Barcroft which was carried out in anaesthetised animals (Chien 1967).

The interaction of anaesthetic agents on the HR and BP response to haemorrhage was largely overlooked until 1967 when observation of the bi-phasic haemodynamic response to blood volume loss were made in conscious animals and in humans (Chalmers, Korner et al. 1967), (Chalmers, Korner et al. 1967), (Vatner and Smith 1974), (Little, Marshall et al. 1989), (Van Leeuwen, Evans et al. 1990), (Shen, Cowley et al. 1991).

Many commonly used anaesthetic agents alter the neural responses to blood loss (Vatner and Smith 1974), (Samar and Coleman 1979), (Adamicza, Tarnoky et al. 1985), (Seyde, McGowan et al. 1985). Pentobarbital sodium, halothane and ketamine attenuate...
or abolish the vasoconstrictor response of the sympathoexcitatory phase during haemorrhage through a central action (Vatner and Smith 1974), (Zimpfer, Manders et al. 1982), (Ebert, Kotrly et al. 1985). Halothane has an additional direct myocardial depressant action and direct vasodilator properties (Vatner 1974). Opioid μ-agonist anaesthetic agents such as fentanyl and alfentanil attenuate the sympathoinhibitory phase but do not affect the sympathoexcitatory phase (Ebert, Kotrly et al. 1988).

Alphaxalone/alphadolone, was reported to have few deleterious effects on the respiratory or cardiovascular systems (Child, Davis et al. 1972). Timms reported that alphaxalone/alphadolone did not interfere with the integrative activities of the forebrain in a way that conventional anaesthetic agents did (Timms 1976). In addition he showed that the visceral changes characteristic of the ‘defence reaction’ could be evoked as a reflex or by stimulation of the amygdala as well as by stimulation of brainstem defence areas in animals anaesthetised with alphaxalone/alphadolone (Timms 1976). Subsequent work indicated that this agent results in a mild reduction in baroreflex sensitivity (Jones and Prys-Roberts 1983). The haemodynamic effects of alphaxalone/alphadolone are dose dependent and with low dose anaesthesia modulations of the baroreflex are preserved and can be readily observed (Sear and Prys-Roberts 1979).

Anaesthetic agents can also influence the size of induced brain injury in experimental models of neurotrauma. For example Baughman and colleagues using a rat model of incomplete cerebral ischaemia found that isoflurane and methohexital improved outcome following moderate ischaemia (Baughman, Hoffman et al. 1990). Studies on the effects of alphadaolone/alphaxalone anaesthesia on cerebral blood flow suggest that
it has no significant effect on cerebral autoregulation (Pickerodt, McDowall et al. 1972). It is associated with a reduction in cerebral metabolism, CBF and ICP and therefore is likely to have some neuroprotective effects following acute TBI (Pickerodt, McDowall et al. 1972), (Rasmussen, Rosendal et al. 1978), (Keaney, McDowall et al. 1978). This neuroprotective effect may limit the extent of neuronal injury following induced mild and moderate TBI. However this would not be expected to alter the observations made in these controlled experimental models.

6.2.3 Haemorrhage Model

Haemorrhage is probably the simplest form of trauma to simulate. However even this form of trauma is studied using numerous distinct models (Majde 2003). Haemorrhagic shock is a very complex condition which involves neural, endocrine, cardiovascular and immune responses as well as cellular adaptation to whole body hypoxia and ischaemia. There are close species correlations between man and the rat in the physiological mechanisms underlying cardiovascular homeostasis (Little, Randall et al. 1984), (Schadt and Ludbrook 1991), (Evans, Ventura et al. 2001).

Animal models are used for two very distinct applications: investigating pathophysiological mechanisms and the assessment of therapeutic interventions (Deitch 1998), (Majde 2003). The focus of the investigations undertaken in this thesis was on pathophysiological mechanisms. Therefore the fixed-volume controlled haemorrhage model was adopted as this model allowed accurate comparisons of HR and MAP responses to progressive haemorrhage between groups.
Uncontrolled haemorrhage is a more common occurrence in the clinical situation than controlled haemorrhage. The basic physiological processes underlying the responses to both controlled and uncontrolled haemorrhage are similar. The use of an uncontrolled haemorrhage model in this investigation would have resulted in significant problems with the experimental design. In uncontrolled haemorrhage the rate and therefore volume of haemorrhage varies. In addition individuals bleed at different rates. Acute TBI may further alter the rate and pattern of blood loss. An uncontrolled model of haemorrhage would therefore have made it very difficult to accurately compare the HR and MAP responses between TBI and sham injured groups. This model would therefore have been inappropriate to use due to the introduction of systematic bias into the results. In addition it would have required the use of a large number of animal subjects which would have been unethical.

6.2.3.1 The use of an additional injury model.

In the clinical setting haemorrhage is frequently accompanied by soft tissue injury (Sarrafzadeh, Peltonen et al. 2001). Evaluating the combined effects of soft tissue injury with haemorrhage in the setting of acute TBI is of clear clinical importance. Soft tissue injury also modifies the response to haemorrhage (Little, Randall et al. 1984). In the investigation into effects of acute TBI on cardiovascular pathophysiology in the trauma setting the important first step was to elucidate the effect of acute TBI on the response to haemorrhage in the absence of soft tissue injury which could potentially confound the interpretation of the results. Having elucidated this effect it would be important to study the effects of acute TBI on the haemodynamic responses in more complex pre-clinical models which would include soft tissue injury (Statler, Jenkins et al. 2001).
6.2.4 Baroreflex assessment technique

A quantitative approach requires a measurement of changes in output resulting from a controlled alteration of input. In the simplest form two recordings are made. If however, as in these experiments, measurements are made of numerous input levels this reduces inaccuracies arising from interpolation between two data points, which would otherwise have resulted in an under-estimation of the reflex sensitivities or failure to detect a change in the reflex sensitivity.

6.2.4.1 The modified Oxford technique

Smyth et al first described a pharmacological technique, also termed the Oxford technique, for testing the baroreflex in man in 1969 (Smyth, Sleight et al. 1969). This technique involves the use of a pressor agent to increase arterial blood pressure 15-20mmHg while recording the reflex changes in heart period or heart rate. The major advantages of this technique are that it is minimally invasive, easy to use and can be conducted in conscious animals and subjects as they are generally unaware of the stimulus. Using this technique baroreceptors are exposed to a natural pulsatile pressure and all of the baroreceptor regions are stimulated.

Angiotensin (the pressor agent initially employed to test baroreflex sensitivity) was replaced by phenylephrine (Bristow, Gribbin et al. 1969) when it became evident that angiotensin could have a direct effect on both the vasomotor centres and the heart (Krasney, Paudler et al. 1966). When used in high doses, phenylephrine can also interfere with the assessment of baroreflex sensitivity by enhancing baroreceptor
stimulation through an increase in smooth muscle tone in the carotid arteries (Peveler, Bergel et al. 1983) and by exerting a small direct positive chronotropic effect (Williamson, Seifen et al. 1994). Repeated testing using this drug can therefore result in a phenylephrine-induced increase in baroreflex sensitivity (Little and Redfern 1981). We limited our investigation of baroreflex activity to short term assessment of the period immediately following acute TBI when the need for compensation for extracranial blood volume loss was most likely to occur. Alteration in baroreflex function remains important at other times in the post injury period but was not considered the main focus of this investigation.

In animals with a high resting HR there are a number of problems with the application of the technique as first described by Smyth et al. (Smyth, Sleight et al. 1969). Because the HR is high in relation to baroreflex latency, many beats occur before the elevated BP results in a lengthening of the heart period. With a rapid elevation of arterial blood pressure the rise in BP is almost complete before the onset of bradycardia (Redfern 1981). This led Redfern to develop a method involving the injection of a pressor agent over a longer time frame to improve the correlation between the elevation of BP and heart period. This technique was modified further by Jones (Jones 1989), who described a rapid infusion phenylephrine pressor test, involving a 60-120 second infusion period. This reduces the total dose of phenylephrine required.

Heart period rather than heart rate is plotted against systolic pressure to assess baroreflex activity. This is because the plot of heart period against systolic pressure is essentially linear over the mid-range of the systolic blood pressure. A change in the slope of the SBP/heart period relationship represents a change in the sensitivity of the
baroreflex and a parallel shift of the slope represents a re-setting of the baroreflex (Smyth, Sleight et al. 1969).

Since the original design of Smyth et al many investigators suggested the use of an additional pharmacological challenge to assess the tachycardic response using agents such as nitroprusside (Cerutti, Barres et al. 1994). It was considered that this gave more information about the operational range of the baroreceptor-heart rate response, as the response to reduction in blood pressure using nitrates was different to that observed with pressor agents. More recent evidence however suggests that nitrovasodilators have significant extra-vascular effects that bias the assessment of baroreflex sensitivity (Casadei and Paterson 2000). In particular, the inhibitory action of NO on baroreceptors activity (Matsuda, Bates et al. 1995), and on cardiac sympathetic neurotransmission (Schwarz, Diem et al. 1995) and signalling (Musialek, Lei et al. 1997) results in depression of the pulse interval response to baroreflex deactivation. The increasing evidence of the importance of NO in modulating cardiac pacemaker activity and autonomic function raises important concerns regarding the use of these agents for testing the arterial baroreflex (Casadei and Paterson 2000).

The main disadvantage of the assessment of baroreflex activity using vasoactive techniques is that injection of the drug affects the afferent activity of number of different receptors in addition to the arterial baroreceptors, e.g. the cardiopulmonary receptors. This produces a complex response resulting from the interaction of reflexes arising from the stimulation of receptors at different sites (Pardini, Lund et al. 1991). Therefore the reflex resulting from such changes in arterial blood pressure should be referred to the baroreflex rather than the baroreceptor reflex where the arterial baroreceptors are stimulated specifically. Whilst the sensitivity of the baroreflex is
determined using this technique it is focused on the cardio-vagal aspect of the response and gives no information about the sympathetic limb of the response (Redfern 1981). Therefore studies on the baroreflex bradycardia provide information about neural control of the heart, but information regarding the reflex control of the vasculature must be obtained separately. Importantly it cannot be assumed that baroreflex bradycardia is a description of the entire baroreceptor reflex. There is evidence that both arms of the reflex response can be affected differently; for example, patients with essential hypertension show a reduced slope of their heart period-arterial blood pressure relationship (Bristow, Honour et al. 1969), whereas the slope of their arterial blood-pressure carotid sinus pressure relationship is increased (Ludbrook, Mancia et al. 1980).

Acute TBI has profound effects on the sympathetic limb of the autonomic system (Atkinson 2000). It is associated with a pressor response which is directly proportional to the size of the induced brain injury (Atkinson 2000). The precise aetiology of this pressor response remains uncertain. Evidence suggests that it is unlikely to be related to raised ICP (Atkinson 2000). It is dependent on the presence of an intact brain stem (Walker 1944), (Atkinson 2000). The catecholamine responses to isolated moderate TBI are markedly potentiated by the presence of extracranial haemorrhage (Yuan, Wade et al. 1991). As TBI also affects the sympathetic arm of the autonomic system it would be important to assess arterial baroreflex function using other modalities such as heart rate variability analysis. The implications of altered baroreflex function on the haemodynamic stability of brain injured patients also require further study.

This model was limited to assessment of the baroreflex in the short term following acute TBI. The focus was on baroreflex function during the period when trauma
patients with co-existing TBI are more likely to be challenged with acute volume loss from peripheral injuries, during which time arterial pressure is normally buffered principally by the arterial baroreflex. Longer term assessment of arterial baroreflex would be an important next step in this investigation. The function of the arterial baroreflex in the context of more complex models of combined TBI and extracranial haemorrhage which is complicated by co-existing soft tissue injury would also form an important basis for future work in this area.

An important outcome of future research must be to refine our current diagnostic tools for the assessment of multiple trauma patients. Non-invasive monitoring may revolutionise the care of the critically ill if data analysis techniques can be validated that identify physiological deterioration in advance of clinical deterioration.
6.3 **Key Findings**

- Acute TBI of moderate severity is associated with a doubling of the risk of death following multi-system trauma (odds ratio 2.08, 95% CI, 1.57 – 2.77)

- Acute TBI of mild and moderate severity is associated with attenuation of the bi-phasic haemodynamic response to haemorrhage. This effect is graded according to the severity of TBI. In the moderate group a 50% early mortality was observed. In the non-survivor subgroup of the moderate TBI group the bi-phasic haemodynamic response was abolished. Paradoxically heart rate and blood pressure were maintained higher for longer during 40% haemorrhage in the subgroup that subsequently died suddenly.

- The use of Shock Index as an indicator of underlying haemorrhage is unreliable in the presence of acute TBI.

- Acute TBI of mild and moderate severity is associated with a marked increase in baroreflex sensitivity immediately after injury. The relative increase in baroreflex sensitivity is closely correlated with the size of TBI.
6.4 CONCLUSIONS

Moderate TBI in isolation is associated with a low mortality rate (< 4.7%). When overall injury severity and age are adjusted for, mortality from multi-system trauma is doubled by the presence of moderate TBI (odds ratio 2.08, 95% CI, 1.57-2.77). Therefore acute TBI of even moderate severity adversely affects survival from multi-system trauma.

Acute TBI was associated with a significant modification of the bi-phasic haemodynamic response to simple haemorrhage. This effect was graded according to the severity of brain injury. The bi-phasic heart rate and blood pressure response was completely abolished in non-survivors. These findings reflect a significant disturbance of cardiovascular homeostasis following acute TBI.

When TBI is combined with haemorrhage Shock Index failed to reflect the trend of increasing blood volume loss. Shock Index is an unreliable indicator of early shock in the presence of acute TBI.

The arterial baroreflex is the one of the principal reflexes involved in the moment to moment control of blood pressure. The effect of acute TBI on the arterial baroreflex was studied using the rapid phenylephrine pressor test. TBI was associated with a sharp increase in baroreflex sensitivity within 10 minutes of induced mild and moderate TBI (125%, 45% respectively). The effects were graded according to the severity of induced brain injury.
In this research, some of the basic interactions of acute TBI and cardiovascular homeostasis have been examined. Future work will need to explore more complex models and interactions in order to enhance our knowledge in this field, thereby leading to improved outcomes for this important patient cohort.
6.5 Future Work

- In this investigation the important two-step comparisons were made evaluating the effects of acute TBI of differing severities on the normal cardiovascular response to haemorrhage. Having characterised the effects of acute TBI on the response to simple haemorrhage it would be important to conduct further research into more complex models of acute TBI and haemorrhage with the addition of other common clinical confounding factors such as soft tissue injury, alcohol, commonly used anaesthetic agents. Within these more complex models it would be important to consider functional imaging studies of injured sites in the central nervous system, in order to correlate the cardiovascular effects with the principal sites of injury.

- Previous research into the effects of injury has indentified that in addition to alteration of the bi-phasic response to haemorrhage, there are changes in the systemic distribution of blood flow, with blood flow diverted away from areas with a low ischaemic tolerance to areas with a higher ischaemic tolerance. It would also be important to investigate the effect of acute TBI on systemic blood flow dynamics.

- Shock Index is considered to be inversely proportional to left ventricular stroke work. Having identified a disturbance of the performance of Shock Index in the presence of acute TBI in this work, it would of interest to study myocardial performance in the presence of acute TBI.
• Future work will require evaluation of alteration of cardiovascular regulation with functional imaging/histology of the central nervous system.

• It would be important to study the integrity of autonomic function using non-invasive tools in both the preclinical and the clinical setting. This should refine our ability to assess acutely injured patients, leading to improved resuscitation and better outcomes in this important group of patients.

• The increased mortality rate in older patients who sustain acute TBI should be investigated in comparative studies in aging rats.
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neurochemical and biomechanical mechanisms." Laboratory Investigation 74(2): 315-42.


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## APPENDIX 1  GLASGOW COMA SCORE

<table>
<thead>
<tr>
<th>Eyes open</th>
<th>Spontaneously .........................4</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>To verbal command ......................3</td>
</tr>
<tr>
<td></td>
<td>To pain ..................................2</td>
</tr>
<tr>
<td></td>
<td>No response ................................1</td>
</tr>
<tr>
<td>Best Verbal Response</td>
<td>Orientated and converses ..............5</td>
</tr>
<tr>
<td></td>
<td>Disorientated and converses ..........4</td>
</tr>
<tr>
<td></td>
<td>Inappropriate words ....................3</td>
</tr>
<tr>
<td></td>
<td>Incomprehensible sounds ...............2</td>
</tr>
<tr>
<td></td>
<td>No response ................................1</td>
</tr>
<tr>
<td>Best Motor Response</td>
<td>Obeys commands ..........................6</td>
</tr>
<tr>
<td></td>
<td>Localises pain ..........................5</td>
</tr>
<tr>
<td></td>
<td>Normal withdrawal ......................4</td>
</tr>
<tr>
<td></td>
<td>Spastic flexion ........................3</td>
</tr>
<tr>
<td></td>
<td>Extension to pain .......................2</td>
</tr>
<tr>
<td></td>
<td>No response .............................1</td>
</tr>
</tbody>
</table>
APPENDIX 2  CALIBRATION OF THE HPLC PUMP

Figure 21 Calibration of the HPLC pump

Relationship between the pressure set at the HPLC pump and the cortical impact pressure. Values are means of triplicate measurements of the peak pressure measured using a pressure transducer. Standard errors were less than 3% of the mean.
APPENDIX 3 DRUGS USED

1-phenylephrine HCL (sigma) 100mg of powder dissolved in 10ml of 0.1M HCL (aq) to give a 1mg⁻¹ stock solution. 1ml of this diluted to 150μg ml⁻¹ with 0.9%w/v NaCl (aqueous) containing heparin (10 IU ml⁻¹).
APPENDIX 4 PUBLICATIONS ASSOCIATED WITH THESIS

Published/in press original articles:


Submitted original articles:


Unexpected Contribution of Moderate Traumatic Brain Injury to Death after Major Trauma

C. Geraldine McMahon, MB, BSc, Anat, FRCSIEd, FFAEM, David W. Yates, MD, FRCS, FFAEM, Fiona M. Campbell, MSc, Sally Holits, MSc, and Maralyn Woodford

Background: The cardiovascular reflex responses to injury and simple hemorrhage are coordinated in the central nervous system. Coincidental brain injury, which is present in 64% of trauma patients who die, could impair these homeostatic responses. The occurrence of hemorrhagic shock in the patient with head injury is also known to increase mortality. Therefore, there is a potential bidirectional interaction between traumatic brain injury and peripheral injury, which would result in an increased mortality when these two injuries coexist. Objective was to test the hypothesis that moderate traumatic brain injury is an independent predictor of outcome in patients with multisystem trauma.

Methods: We carried out an analysis of the UK Trauma Audit Research Network Database. Moderate traumatic brain injury was defined as an Abbreviated Injury Scale score of 3.

Trauma continues to account for more deaths in the first 4 decades of life than all other diseases combined and is the third most common cause of deaths in all age groups. Head injuries remain the most important single injury contributing to mortality and morbidity after accidental injury. Laboratory evidence suggests that traumatic brain injury adversely affects cardiovascular compensation for simple hemorrhage. The effects of brain injury on the responses to soft-tissue injury are unknown. Many trauma patients have a combination of all three insults; therefore, it is important to identify any interaction between acute central nervous system injury and peripheral injury that could modify survival in multisystem trauma.

Analysis of the UK Trauma Audit and Research Network Database reveals that 21% of all those who reach the hospital alive have a traumatic brain injury but this rate increases to 64% in those who subsequently die. Some of these deaths are caused by the severity of the primary brain injury itself; however, it is hypothesized that cerebral damage may also impair the systemic response to extracranial injury. This would result in an increase in mortality and morbidity from peripheral injury and secondary brain damage.

To examine the effect of traumatic brain injury on outcome after multiple trauma, an analysis was carried out by using the extensive database of the UK TARN. Factors that are known to affect outcome, i.e., age, injury severity, and the presence of preexisting medical conditions, were included in the analysis (F. M. Campbell, D. W. Yates, personal communication).

The UK TARN database (a development of the Major Trauma Outcome Study) is the product of a multicenter prospective cohort study and contains descriptive data on injury severity and outcome after trauma. At the time of analysis, the database contained data on more than 42,000 patients collected between 1989 and 1995 from over 130 hospitals in the United Kingdom.

PATIENTS AND METHODS

Injuries were coded by using the 1990 revision of the Abbreviated Injury Scale (AIS). Moderate traumatic brain injury was defined as an AIS score of 3 "internal organ" injury in the "head injury" section of the coding manual. This rating consists predominantly of small cerebral contusions (≤30 mL, 4 cm diameter; ≤5 mm midline shift) as identified by computed tomographic scan, magnetic resonance imaging scan, or by autopsy. Traumatic subarachnoid hemorrhage (mild degrees of brain swelling) were also included in the group (AIS score < 3). Injuries to the major intracranial vessels (laceration, thrombosis, and traumatic aneurysms) are coded separately from internal organ damage. They are also severe injuries and score more than AIS score of 3. Therefore, they were not included in this study group, as this inclusion would have led to the selection of an overall higher severity of intracranial injury, which was not the subject of this study. Isolated AIS score of 3 brain injuries were associated with a mortality rate of less than 4.2%.

The study population included 2,717 patients with multisystem injury: 378 patients had a moderate brain injury with peripheral injury, and 2,339 patients had extracranial injury alone. Mortality rates for both groups were compared at increasing injury severity.

Results: Moderate brain injury alone was associated with a mortality rate of 4.2%. However, when combined with extracranial injury, the risk of death was double that attributable to extracranial injury alone (odds ratio, 2.08; 95% confidence interval, 1.57–2.77).

Conclusion: This study confirms that the coexistence of moderate traumatic brain injury with extracranial injury is associated with a doubling of the predicted mortality rate throughout the injury severity ranges studied.

From Hope Hospital, Salford (C.G.M., D.W.Y., F.M.C., M.W.), and Lancaster University (S.H.), United Kingdom.

Address for reprints: C. Geraldine McMahon, MB, BSc, Anat, FRCSIEd, FFAEM, University of Manchester, Clinical Sciences Building, Hope Hospital, Salford, M6 8HD UK.
The study population was limited to patients who sustained blunt trauma with an Injury Severity Score (ISS) between 16 and 50. These parameters were chosen to reflect the range of injury severity for which traumatic brain injury could be expected to affect mortality. The brain-injured group had an AIS score of 3 brain injury and extracranial injury contributing to their overall ISS. These patients may have had other associated head injuries with AIS score of 2 or less (e.g., associated skull fracture). The control group was patients with extracranial injury only. Patients with scalp and skull injuries were not included in the control group to avoid the presence of unrecognized associated brain injury in this group. Only cases with complete data were used. However, this method resulted in the exclusion of less than 0.01% of all potential cases.

The probability of death was modeled by using logistic regression adjusting for ISS and age. In this study, we chose not to include the Revised Trauma Score (RTS) as used in TRISS because it is heavily dependent on the Glasgow Coma Scale score. An alteration of Glasgow Coma Scale score in the patient without head injury is secondary to a different mechanism (e.g., severe hypoxia, hypovolemia, or intoxication). The inclusion of Glasgow Coma Scale score in this analysis, therefore, would be inappropriate. However, Bull and Dickson have shown that ISS and age together are good prognostic indicators of survival. Recent evidence from the UK TARN database shows that the effect of age on survival is better represented by categories rather than a single step at the age of 55 years as used by TRISS (F. M. Campbell, D. W. Yates, personal communication). Therefore, we used injury severity, age (<16, 16–25, 26–40, 41–55, 56–65, 66–75, 76–85, >85 years), and the presence of head injury to analyze the effect of traumatic brain injury on outcome. This was achieved by using logistic regression in SPSS for Windows version 6.3.

RESULTS

The group with brain injuries consisted of 378 patients, and the control group consisted of 2,339 patients. Analysis of the distribution of injuries in both groups revealed an increased incidence of facial injuries in the group with head injuries. However, exclusion of patients with facial injuries did not significantly change the results of the analysis. There were slightly fewer chest and abdominal injuries in the group with head injuries, which may indicate a potential underestimation of the effect of brain injury on outcome. This was achieved by using logistic regression in SPSS for Windows version 6.3.

![Figure 1](image1.png)


<table>
<thead>
<tr>
<th>Region</th>
<th>Moderate Traumatic Brain Injury (%)</th>
<th>No Traumatic Brain Injury (%)</th>
<th>χ² (p Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck</td>
<td>8 (2.1)</td>
<td>27 (1.2)</td>
<td>2.37 (0.124)</td>
</tr>
<tr>
<td>Face</td>
<td>103 (27.2)</td>
<td>211 (9)</td>
<td>105.8 (&lt;0.0001)</td>
</tr>
<tr>
<td>Chest</td>
<td>231 (61.1)</td>
<td>1,585 (67.8)</td>
<td>6.50 (0.011)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>93 (24.6)</td>
<td>847 (36.2)</td>
<td>19.38 (0.0001)</td>
</tr>
<tr>
<td>Extremities</td>
<td>305 (80.7)</td>
<td>1,360 (58.1)</td>
<td>69.70 (&lt;0.0001)</td>
</tr>
<tr>
<td>Skin</td>
<td>182 (48.1)</td>
<td>991 (42.4)</td>
<td>4.43 (0.035)</td>
</tr>
</tbody>
</table>

* n = 378.
* n = 2,339.


Data on premorbid medical conditions were only available for 40% of patients. Our analysis demonstrated that the compounding effect of preexisting medical conditions was large. However, this effect did not reach statistical significance; the odds ratio adjusted for ISS and age was 2.18 (95% confidence interval [CI], 0.70 to 6.81). The wide CI is most likely to be secondary to the large amount of missing data (Fig. 4 and Table 4).

The logistic model allows analysis of the independent
effect of moderate traumatic brain injury on the probability of death when other significant variables (ISS, age) are accounted for (Fig. 5). This graph demonstrates the additional

increase in the probability of death occurring in the presence of moderate traumatic brain injury, the odds ratio being 2.08 (95% CI, 1.57–2.77). This finding represents a doubling of the risk of death in patients with moderate traumatic brain injury as a component of multisystem trauma.

One hundred seven of the moderate brain injury group (28%) and 438 of the control group (20%) were transferred between hospitals for further care. When these patients were excluded from the study, the results of the comparisons were unaltered (odds ratio, 2.21; 95% CI, 1.60–3.07).

**DISCUSSION**

Head injury both with and without extracranial trauma remains the single most important factor contributing to death and disability after accidental injury. Severe head injury obviously has a high associated mortality rate; however, this study suggests that even modest head injury when combined with extracranial injury has a detrimental effect on survival.

The validity of this study is critically dependent on the analysis of the UK TARN database. Because injury severity is a major determinant of outcome, it is essential that injury severity is measured accurately. Misclassification may result from either deficiency in the scoring system or inter-rater variation. Injury severity scoring for the UK TARN database is carried out at the North Western Injury Research Centre by a small number of trained staff, thereby reducing inter-rater variation (intraclass correlation coefficient between UK TARN staff is 0.97 [95% CI, 0.95 to 0.98]). An underscoring of the severity of these injuries by the AIS could account
for the increased mortality associated with the combination of moderate brain injury and extracranial injury. However, analysis showed little variation in outcome for isolated AIS score of 3 injuries in the other main body regions.

The multicenter nature of the study could influence interpretation of the findings if very significant variations in the pattern of patient care were identified in a small group of contributing hospitals. Previous analysis of the UK TARN database has shown a wide spread of Ws scores (differences between actual and predicted survival) across hospitals, which cannot be explained by either the volume of trauma handled or the presence of a specialist neurosurgical unit. However, these differences are spread broadly across the 130 hospitals studied with no center being sufficiently exceptional to be able to skew the results.

Analysis of outcome of patients injured at different ages demonstrated an apparent increased mortality in the 26- to 40-year age groups. This may have been a spurious effect; however, it is possible that alcohol or drug intoxication, well-known contributory factors, in accidents involving young people, may have contributed to this increase. Alcohol is well known to potentiate the effects of trauma on central nervous system damage. Recreational drugs may increase the damage occurring after brain injury by neurotoxic and pyrexial effects. The increased incidence of pre-existing medical conditions and associated polypharmacy in the elderly group would result in impairment of normal compensatory mechanisms and, therefore, be an additional factor affecting outcome.

It has been suggested that failure to recognize or adequately treat hemorrhage is a contributory factor in 60% of "preventable" trauma deaths for which there is no head injury. This finding could be more important in patients with brain injuries, because there is clinical and laboratory evidence that traumatic brain injury adversely affects cardiovascular compensation for hemorrhage.

Trauma victims commonly suffer at least two insults, hemorrhage and tissue injury, each of which generate its own pattern of reflex response, which interacts to yield a third pattern of response. Progressive "simple" hemorrhage (in the absence of major tissue damage) produces a biphasic response: an initial tachycardia and increase in vascular resistance, whereas blood pressure is maintained by the baroreflex. This condition is followed by a reflex bradycardia, vasodilatation, and hypotension mediated by a depressor reflex of unknown origin. The activity of both of these reflexes is modified centrally by the presence of nociceptive afferent impulses arising from damaged peripheral tissues. There is experimental evidence that this interaction is detrimental.

The effects of direct brain injury on cardiovascular reflex activity is unknown, although preliminary experimental studies have shown an early and sustained increase in parasympathetic activity in rats subjected to moderate traumatic brain injury. This may have a significant impact on the cardiovascular responses to hemorrhage and implications for survival.

Evidence also exists that hemorrhagic shock associated with neurotrauma induces a secondary brain injury, which may in turn alter cardiovascular control mechanisms. Cerebral oxygen delivery and consumption remain depressed in these patients, despite restoration of systemic hemodynamics.

Therefore, there is evidence of a potential bidirectional interaction between traumatic brain injury and extracranial injury. Traumatic brain injury adversely affects compensation for hemorrhage. Peripheral injury, when associated with hypotension, causes secondary brain injury. It is possible that a reverberating loop of "negative interaction" may exist when these two injuries are combined.

In summary, an association between the increased mortality rate and the presence of moderate traumatic brain injury has been demonstrated. The precise pathophysiological nature of this relationship is unclear; the pathways involved are likely to be complex. Comparison of these appropriately matched populations shows that moderate traumatic brain injury has an adverse effect on survival after multiple trauma. Further work to improve our understanding of this interaction is essential to improve resuscitation and treatment measures and, thereby, reduce the unacceptably high mortality and morbidity associated with this silent epidemic.

REFERENCES


Acute traumatic brain injury (TBI) is the most important single injury contributing to mortality and morbidity following major trauma (1, 2). There is evidence that even moderate TBI, which in isolation is associated with a very low mortality rate (<4.7%), doubles the expected mortality and morbidity in the multiply injured patient (3).

Extracranial hemorrhage and TBI frequently coexist in multisystem trauma victims (4). Clinical and laboratory evidence suggests that TBI adversely affects cardiovascular compensation for hemorrhage (5, 6). The precise mechanisms underlying these adverse effects are incompletely understood, and further investigation is required if outcomes are to improve. Additionally, it has been suggested that failure to recognize or adequately treat hemorrhage is a contributory factor in up to 90% of "preventable" trauma deaths, where there is no head injury (7). This is also likely to be a problem in brain-injured patients, particularly if the acute homeostatic response to hemorrhage is modified.

Trauma victims commonly suffer at least two extracranial insults: hemorrhage and tissue injury, each of which generates its own pattern of cardiovascular homeostatic reflex response, which interact to yield a third pattern of response. The initial investigations into the cardiovascular responses to progressive hemorrhage carried out by Barcroft in the 1940s showed that progressive "simple" hemorrhage (in the absence of major tissue damage) produced a biphasic heart rate (HR) and mean arterial blood pressure response (MAP). In this stage the sensitivity of the baroreflex has increased (10). This second phase is not due to a failure of the baroreflex, as it has been shown that at this stage the sensitivity of the baroreflex is increased (10). This second phase has been attributed to the recruitment of one or more reflexes (11). However, the pre-
Acute traumatic brain injury (TBI) is a significant cause of morbidity and mortality worldwide. The effects of acute TBI on cardiovascular responses to progressive simple hemorrhage are unknown. There is evidence of autonomic uncoupling in patients with severe brain injury (13). Therefore, to allow physiologic variables to return to baseline before the induction of hemorrhage, a further period of 10 min was allowed to elapse. Baseline physiologic measurements of HR, blood pressure, and respiratory rate were then captured as control data for the hemorrhage study. Fifteen minutes following the acute TBI, animals underwent simple hemorrhage of 40% blood volume by methods described. Physiologic measurements were made after withdrawal of each aliquot of blood and at 15-min intervals until 60 min after TBI. A computerized physiologic data acquisition system (Fastdat, Lectromed, UK; real-time data capture, display, and analysis system) was used. Blood gas analysis was subsequently performed on each aliquot of blood (Arkray, Denmark) (Fig. 2). Animals were killed humanely using a lethal injection of sodium pentobarbital (at the end of the posthemorrhage observation period). Postmortem examination of the brains was performed to determine the level of right parietal cortex involved in the injury model (14).

Lateral Fluid Percussion Model
A cranial window was performed stereotactically at the level of the right parietal cortex (3.5 mm anterior to and 6 mm above the interaural line) leaving the dura intact. Teflon tubing (2 mm inner diameter) was placed over the dura and sealed in situ using methacrylate cement. This was connected through a fluid-filled system to an HPLC pump (SFF100 Spectroflow, Kratos Analytical Instruments, UK). The HPLC pump was used to deliver a predetermined level of fluid percussion pressure, which was monitored by the lateral cortex using an electronically controlled solenoid valve (RS, UK). Thus, a brief pulse (20 ms) of sterile water was applied to the lateral cortex using an electronically controlled solenoid valve (RS, UK). This pulse was applied to induce a brief period of apnea, hypertension, and bradycardia (20, 21). Therefore, to allow physiologic variables to return to baseline before the induction of hemorrhage, a further period of 10 min was allowed to elapse. Baseline physiologic measurements of HR, blood pressure, and respiratory rate were then captured as control data for the hemorrhage study. Fifteen minutes following the acute TBI, animals underwent simple hemorrhage of 40% blood volume by methods described. Physiologic measurements were made after withdrawal of each aliquot of blood and at 15-min intervals until 60 min after TBI. A computerized physiologic data acquisition system (Fastdat, Lectromed, UK; real-time data capture, display, and analysis system) was used. Blood gas analysis was subsequently performed on each aliquot of blood (Arkray, Denmark) (Fig. 2).

Animal Groups
The study was conducted on terminally anesthetized male Wistar rats of the Porlon strain weighing 240-290 g, kept since weaning on a 12-hr light/dark cycle and fed on feedway standard rat and mouse diet (B & K Universal, UK). The effects of acute TBI, induced using the LFP brain injury model (14), on the cardiovascular response to hemorrhage were determined.

The present investigation was in accordance with the Animal (Scientific Procedures) Act 1986, which encompasses the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (17).

Surgical Preparation
Anesthesia was induced using isoflurane (Abbott Laboratories, NC, USA) in a 1:1 mixture of oxygen (F.3.0, F.0, 0.5) in an anesthetizing chamber until cessation of spontaneous movement, loss of the righting reflex, absence of blink reflex, and withdrawal to a noxious stimulus (pinch test applied to the foot). Surgical anesthesia was maintained using 2% to 3% isoflurane administered via a mask. The lateral tail vein and ventral tail artery were cannulated using polyethylene cannules (PEG and JRG, respectively). Portex, Ltd. (UK). Both cannulae were perfused with heparinized saline (1000 IU/ml). An arterial catheter was used to measure arterial blood pressure using a strain gauge manometer (Sonosnor 890, Sonosnor, Norway). Electrocardiogram leads were placed subcutaneously on the ventral surface of the animal to record the electrocardiogram, from which the HR was measured. A neonatal respiratory monitor was attached externally to the chest wall and connected to a transducer to monitor respiratory movements. Rectal temperature was monitored with a thermocouple inserted 6-8 cm past the anal sphincter into the colon and was maintained at 37.8 ± 0.5°C (normal temperature for the rat) using a fan, blanket, and overhead heating lamp. On completion of the surgical procedure, the isoflurane and N20 were discontinued. Anesthesia was maintained using continuous intravenous infusion of alfentanil/alfadalone (Saffan, Pitman-Moore, UK; 18-20 mg/kg/hr, intravenously) using an infusion pump (Harvard 22, Harvard Apparatus, UK). This rate of infusion allowed a mild withdrawal and a 10-mm Hg increase in blood pressure in response to a pinch test applied to the Achilles tendon. This anesthetic agent was chosen because it preserves the arterial baroreceptor reflex and the response to hemorrhage (18). Animals spontaneously breathed oxygen-enriched air. After completion of initial surgical procedures, the animal was transferred to a stereotactic frame. A minimum of 60 min was allowed to elapse before physiologic data were recorded to allow an adequate washout period of isoflurane.
Figure 1. Study protocol. LFP, lateral fluid percussion.

Figure 2. Mean (±SEM) of PaO₂, PaCO₂, arterial base excess (ABE), and pH for control, mild, and moderate traumatic brain injury groups with progressive hemorrhage of 40% blood volume.

carried out on all animals immediately following the procedure.

Statistical Methods

Means and standard errors of HR and MAP are presented by volume of hemorrhage, for control, mild, and moderate TBI groups. Baseline data are presented by group using mean and SEM. Analysis of variance (ANOVA) was used to test for any changes in mean values between the three groups.

To determine whether the effects of brain injury and hemorrhage on HR and MAP by volume of hemorrhage were statistically significant, a two-way repeated-measures ANOVA was undertaken to examine differences between the groups with repeats for volume of hemorrhage (12 measurements) within animals. The trend analysis considered two models: 1) assuming a linear association of HR and MAP with volume of hemorrhage; and 2) assuming a linear and quadratic association. The quadratic time variable allows for any curvature in the relationship between HR or MAP and volume of hemorrhage. A test for interaction of group (injury or control) by volume of hemorrhage (linear and quadratic) was used to determine whether there were significant differences in the trend lines between injury and control groups.

In the moderate TBI group, a two-way repeated-measures ANOVA was performed to compare the trends for mean HR and MAP (with repeats for different volumes of hemorrhage) comparing surviving and nonsurvivors.
Table 1. Physiologic measurements after traumatic brain injury (TBI) and before hemorrhage for control, mild, and moderate TBI groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Mild (n = 8)</th>
<th>Moderate (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paco2, mm Hg</td>
<td>39.2 (30.9,35.4)</td>
<td>50.8 (48.0,53.6)</td>
<td>53.4 (49.2,57.5)</td>
</tr>
<tr>
<td>Paco2, mm Hg</td>
<td>22.0 (17.1,27.1)</td>
<td>26.2 (19.1,32.0)</td>
<td>26.1 (17.7,33.4)</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.33 (7.31,7.35)</td>
<td>7.31 (7.32,7.35)</td>
<td>7.33 (7.30,7.34)</td>
</tr>
<tr>
<td>Hb</td>
<td>12.18 (12.01,12.74)</td>
<td>11.61 (10.68,12.34)</td>
<td>12.63 (11.72,12.35)</td>
</tr>
<tr>
<td>ABE, mmol/L</td>
<td>0.01 (0.29,1.54)</td>
<td>0.61 (0.12,1.35)</td>
<td>1.0 (0.02,6.00)</td>
</tr>
<tr>
<td>Temp, °C</td>
<td>37.69 (37.33,37.83)</td>
<td>37.56 (37.33,37.79)</td>
<td>37.61 (37.52,37.79)</td>
</tr>
<tr>
<td>%body weight</td>
<td>251.8 (219.6,260.5)</td>
<td>250.6 (247.2,253.1)</td>
<td>251.4 (246.6,256.2)</td>
</tr>
<tr>
<td>Apnea, mean %</td>
<td>0 (0.0)</td>
<td>3.1 (0.5,5.0)</td>
<td>13.6 (7.8,19.5)</td>
</tr>
</tbody>
</table>

Statistical Power of the Study

With a sample of eight animals having repeated data on 12 occasions, the study had sufficient power (80%) at a 5% level of significance to detect a difference of 1 sec in HR or MAP between any two groups (e.g., moderate and control) in the study.

RESULTS

Baseline Results

Following acute TBI, a brief period of apnea was observed. There were significant differences between groups in the duration of apnea induced by the fluid percussion injury. No apnea was observed in the control group. In mild TBI, the duration of apnea was 3.1 ± 1.1 msecs compared with 13.6 ± 2.5 msecs (p < .001) following moderate TBI.

In mild TBI, there was transient elevation of systolic blood pressure of 15 ± 11 mm Hg compared with 41 ± 6 mm Hg (p = .002) in moderate TBI following the fluid percussion injury. No pressor response was observed following sham injury.

There were no significant differences between groups in the initial baseline values of body weight, HR, MAP, or arterial blood gases at the control time interval. Measured 10 mins after acute TBI, and immediately before the commencement of the hemorrhage phase. Heart rate was observed to be lower in the moderately injured animals before undergoing hemorrhage, but this did not reach statistical significance (Table 1).

Arterial blood gas analysis during the hemorrhage period revealed no significant differences between the groups (Fig. 2). The Paco2 fell with progressive hemorrhage (linear trend, B = -0.79, SE = 0.10, p < .001). No hypoxic episodes occurred in the study animals.

Postmortem evaluation of the moderate TBI group revealed macroscopic evidence of contusion on the surface of the ipsilateral cortex with small amounts of subarachnoid hemorrhage as described in other reports (22). The mild and control animals had no macroscopic evidence of brain injury.

Mortality

No mortality was observed in the control or mild TBI groups. Four (50%) of the moderate TBI animals died suddenly after the completion of hemorrhage, at 45, 55, 60, and 90 mins.

Figure 3. Mean (±SD) mean arterial pressure (MAP) and heart rate (HR) for increasing percent volume of hemorrhage in mild traumatic brain injury (TBI), moderate TBI, and control groups. In the moderate TBI group, eight animals were alive at 45 mins and five animals were alive at 60 and 90 mins.

Table 2. Postmortem findings after hemorrhage in control, mild, and moderate TBI groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mild</th>
<th>Moderate</th>
</tr>
</thead>
<tbody>
<tr>
<td>%body weight</td>
<td>251.8</td>
<td>252.6</td>
<td>253.4</td>
</tr>
<tr>
<td>%Paco2</td>
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<td>4.5</td>
<td>4.3</td>
</tr>
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<td>%PacO2</td>
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<td>20.6</td>
</tr>
<tr>
<td>%Arterial pH</td>
<td>7.3</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>%Hb</td>
<td>12.2</td>
<td>12.1</td>
<td>12.3</td>
</tr>
<tr>
<td>%ABE</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Figure 4. Mean (±SEM) mean arterial pressure (MAP) and heart rate (HR) for increasing percent volume of hemorrhage in mild traumatic brain injury (TBI), moderate TBI, and control groups. In the moderate TBI group, eight animals were alive at 45 mins and five animals were alive at 60 and 90 mins.
Heart Rate and Blood Pressure Responses to Hemorrhage

Control Group. In the control animals, progressive hemorrhage produced a biphasic response of HR and MAP (Fig. 3). HR showed an initial increase until 17.5% ± 1.6% blood volume loss occurred. The maximum increase in HR was 24 ± 4.2 beats/min, which indicated a significant change from baseline ($p < .01$). However, as the severity of hemorrhage increased beyond 17.5% of total blood volume, a marked bradycardia associated with a precipitous decrease in blood pressure took place (Fig. 3). The maximum drop in HR was 107 ± 7.5 beats/min, which indicated a significant change from baseline ($p < .01$).

Mild Head Injury Group. The HR and MAP response by volume of hemorrhage was compared between mild TBI and control groups. There was a small delay in the onset of bradycardia. This divergence in response became significant at 27% blood volume loss ($p = .044$). However, the overall biphasic pattern of response was maintained. This was confirmed in the quadratic analysis of the HR and MAP response by volume of hemorrhage. A repeated-measures ANOVA showed a significant linear ($\beta_1 = 14.55$, $se = 2.7$) and quadratic ($\beta_2 = -1.98$, $se = 0.21$) association of volume of hemorrhage on HR ($p < .0001$) for both groups (Fig. 2). However, the biphasic pattern of HR response did not differ significantly between the mild TBI and control groups.

Similarly, the pattern of MAP response overall did not differ significantly between the mild TBI and control groups. There was a significant quadratic ($\beta_1 = -0.61$, $se = 0.11$) association of volume of hemorrhage on blood pressure ($p < .0001$) for both groups (Fig. 3). The largest difference between the mild and control groups was observed at 26.7% volume hemorrhage (difference = 55.2 ± 10.8), but the pattern of MAP response overall did not differ significantly between the mild and control groups. Therefore, while the trend of response for HR and MAP was similar in both groups, the depressor phase of the response was delayed in the mild TBI group (Fig. 3).

Moderate Head Injury Group. In contrast with mild brain injury, the pattern of HR and MAP response was altered after moderate brain injury. Both HR and MAP were initially lower in the prehemorrhage phase following moderate brain injury. The HR response to hemorrhage in moderate TBI animals was significantly different than that of control animals (Fig. 4). The expected biphasic HR response was markedly attenuated after moderate TBI. A divergent pattern of HR response was identified between the moderate TBI and control groups, with a higher HR response observed in the moderately injured animals (38 ± 8.6 beats/min) until 23% volume of hemorrhage was reached when the trend was reversed. The maximum fall in HR was 51 ± 15 beats/min at 31% blood volume loss compared with 107 ± 7.5 beats/min in the control group, which occurred at 17.5% blood volume loss. The repeated-measures ANOVA showed a significant linear ($\beta_1 = 10.36$, $se = 2.89$) and quadratic ($\beta_2 = -0.43$, $se = 0.22$) trend of volume on mean HR ($p < .01$). There was a significant difference in this trend of HR response observed between the moderate TBI group and the control group (test for interaction $p < .001$) and between the moderate and mild TBI groups (test for interaction between groups, $p < .0001$).

Similarly, the MAP response to hemorrhage was also markedly altered following moderate brain injury. There was a significant difference in overall linear ($\beta_1 = -3.62$, $se = 1.47$, $p = .015$) and quadratic trend ($\beta_2 = -3.9$, $se = 0.11$, $p < .001$) between the groups, across volume of hemorrhage. This indicates that the pattern of blood pressure response to progressive simple hemorrhage was significantly different between the moderate TBI and the control groups.
significantly attenuated the depressor response as nol significantly different as a survival analysis investigation, following the observed 50% mortality rate in the moderate TBI group, a preliminary subgroup analysis of this group was undertaken. Animals were stratified into survivors and nonsurvivors. Clear differences in the HR and MAP response to hemorrhage between the survivors and nonsurvivors were identified. Survivors still showed a biphasic hemodynamic response that was modified compared with controls, that is, the onset of bradycardia and hypotension was delayed until the response was more pronounced than in the mild TBI group. In nonsurvivors, the biphasic HR and MAP response was abolished (Fig. 5). At the end of hemorrhage, MAP was significantly higher in the nonsurvivors compared with either the survivors (difference = 42.0 mm Hg; 95% confidence interval 17.7, 67.3; \( p = 0.007 \)) or the control group (difference = 42.9 mm Hg; 95% confidence interval 23.4, 62.4; \( p = 0.002 \)). When HR and MAP responses of nonsurvivors vs. survivors were compared using repeated-measures ANOVA, there was a significant difference in HR and MAP by volume of hemorrhage (Fig. 4; test for interaction, \( p = 0.029 \), \( p = 0.013 \), respectively). The mean (\pm ) apnea period in those who died (19.90 ± 2.18 sec) compared with survivors (10.58 ± 4.27 sec) was not significantly different (\( p = 0.26 \)). Postmortem evaluation of the moderate TBI group revealed no significant macroscopic difference between animals that died compared with those that survived.

**DISCUSSION**

Assessment and management of the acutely injured patient depend on the assumption that all homeostatic reflexes are functioning normally. The principal finding of this study was that acute TBI modified the cardiovascular response to hemorrhage and this effect was graded with respect to severity of TBI. After mild TBI the biphasic response to blood loss was maintained, but the onset of the second, depressor phase of the response to hemorrhage was delayed. Moderate TBI significantly attenuated the depressor response to hemorrhage such that MAP was maintained higher for longer in the moderately injured group compared with either the control or the mild TBI group. The effects of acute TBI on the reflex responses to hemorrhage have not been previously elucidated in either humans or rodent models. To the best of our knowledge, this is the first report on the effects of acute TBI on the biphasic response to hemorrhage.

**Experimental Model**

Several models of hemorrhage are used in experimental studies, including models of controlled blood loss to either fixed volume of fixed pressure end points, or uncontrolled hemorrhage, and the choice of model is dictated by the hypothesis being tested. The principal hypothesis being tested was that acute TBI would modify the pattern of response to hemorrhage; consequently, the model of choice was a fixed-volume, controlled hemorrhage model (23). This type of model is viewed as the most appropriate when investigating potential modulations of the reflex response to blood loss, where it is essential that the degree and rate of blood loss are the same between experimental groups (24). An uncontrolled hemorrhage model (25, 26) would be inappropriate in this type of study because the altered pattern of blood loss could introduce a systematic bias between groups. It would not be possible to determine whether any difference in pattern of response to blood loss was due to, or resulted from, the difference in profile of blood pressure change during hemorrhage. This investigation was therefore conducted in line with previously published work in this area using a controlled fixed-volume hemorrhage model (8, 11, 27-30). Our results confirm that our model reflects the sequential activation of these reflexes as illustrated by the responses observed in the sham injury group.

In multiple trauma patients, extracranial blood loss is often accompanied by soft tissue injury. Soft tissue injury modifies the reflex response to hemorrhage, resulting in an attenuation of phase 2 of the biphasic HR and MAP response (10, 12, 27). In addition, the response to soft tissue injury may interact with the response to TBI. Although investigating a three-way interaction among hemorrhage, TBI, and soft tissue injury is of clinical importance, interpreting the results would be difficult without prior knowledge of the two-way interaction between the response to blood loss and acute TBI. The present study has therefore focused on this first step, which provides a sound basis to investigate models of greater clinical complexity in the future, which could ultimately include a number of factors, such as coexisting soft tissue injury, the occurrence of secondary insults, intoxication, age, and other factors that influence the reflex responses (12, 31, 32).

The complexity of the clinical setting does not detract from our observation that the cardiovascular response to hemorrhage are modified following acute moderate TBI in the rodent model. There is evidence of close species correlation in the cardiovascular reflex responses to hemorrhage observed in the present study compared with studies in humans, other large animals, and rodents (8, 9, 33, 34).

The choice of anesthetic used in this model is critically important. Anesthetic agents can modify the baroreflex and the injury-induced modulations of baroreflex function. Hence, much of the original experimental work carried out on cardiovascular reflex activity was done using conscious animals. We used low-dose intravenous alphadoline/alphaxalone anesthesia as it has been shown to preserve baroreflex function and the effects of injury modulations (nociceptive afferent activity) on the baroreflex (8, 23, 25).

The LFP model of brain injury in the rat is one of the most widely used experimental models of brain injury because of its construct validity with closed head injury in humans (16, 20, 36). This model of brain injury enabled reproducible severities of brain injury to be produced. The histologic changes resulting from this brain injury model include a well-defined ipsilateral cortical and ipsilateral hippocampal and amygdaloid injury (16, 22). Other aspects of molecular, vascular, and behavioral correlates of different severities of human brain injury are closely replicated in this model (18).

**Principal Findings**

The biphasic HR and MAP response to hemorrhage observed in the control group was consistent with previous investigations reported in humans (8, 39) and animal studies (9, 34). Following mild TBI, the biphasic pattern of HR and MAP response was maintained, but the onset
Animals were stratified into survivors and nonsurvivors following the observation that the biphasic response was markedly attenuated in the group of survivors, as it was observed in decerebrate animal models of brain injury (39). In our study we observed an apneic response that closely reflected the severity of induced brain injury. This is consistent with other laboratory data (38, 40). Dysfunctional respiration and apnea occurring at the time of TBI can result in increased intracranial pressure (38). Hypocapnia inhibits baroreflex-induced bradycardia (41). However, this was not a factor in our model as supplemental oxygen was delivered throughout the experimental period. Therefore, this would not account for the observed modulation in the vagally induced bradycardia.

Observation of the trend in Paco2 levels with progressive hemorrhage showed the expected fall in the Paco2 levels in all groups. No significant difference was observed in the pattern of response between the three groups. While this was not a significant difference, it was observed modulation in the vagally mediated bradycardia.

Limitations of the Study

Our study design was specifically focused on the assessment of the biphasic cardiovascular response to hemorrhage. It was conducted as a survival analysis study. This limited the power of the subgroup analysis of survivors and nonsurvivors. Because this study was conducted as a nonsurvival experiment, detailed histologic analysis of the injured brain was not performed. This would have required survival of the animals for a number of days (16). Postmortem evaluation of the moderately injured animals revealed no macroscopic difference between survivors and nonsurvivors. The precise cause of death in nonsurvivors was not accurately determined. They had similar preinjury observations: an abrupt onset of a brief period of dysfunctional respiration, bradycardia, and hypotension resulting in asystolic cardiac arrest within seconds of onset of dysfunctional respiration. This laboratory study identifies an acute disturbance in the neural responses to acute hemorrhage in the rodent model using well-validated models of closed head injury and simple hemorrhage. While there is evidence of close species correlations in the pathophysiology of TBI and the physiologic responses to hemorrhage, these findings need to be validated in the clinical setting.

We have limited our investigation to evaluating the effect of acute TBI on the biphasic hemodynamic response to hemorrhage.
ACKNOWLEDGMENTS

We thank Professor Rod Little, head of department, for advice and guidance, and Hazel Marshall, senior laboratory technician, for support and technical assistance during the laboratory work.

REFERENCES

2. Kincaid JP, MacArthur DL. Epidemiology as a guide for advice and guidance, it is important not to be afraid of the effects of acute TBI on the reflex responses to hemorrhage. Further investigation into the effects of acute TBI on the response to hemorrhage confounded by coexisting soft tissue injury is an important next step in improving our understanding of the pathophysiology of acute TBI on cardiovascular regulation.

CONCLUSIONS

We have shown in a laboratory model that acute TBI significantly modifies the normal cardiovascular reflex responses to progressive simple hemorrhage. This effect is graded according to the severity of the induced brain injury. This modification was observed even in the presence of mild TBI. Moderate TBI was associated with a marked alteration in HR and MAP response to hemorrhage. Acute disturbance in cardiovascular regulatory control was induced by profound unresponsiveness to hemorrhage. This may in part explain the unexpectedly high mortality rate seen in patients who sustain acute TBI as a component of multisystem trauma (1, 3). Currently there are no readily available clinical tools to assess the integrity of autonomic function in the acute clinical setting. This may be a very important factor influencing the approach to resuscitation and choice of anesthetic agents used in the clinical care of these patients. Further clinical and laboratory research in this area is needed to improve our understanding of the cardiovascular pathophysiology in brain-injured patients and thereby translate into improved outcomes in this group.
circular adjusViTicnts to hemorrhage in the cat. Acta Physiol Scand 1970; 8":
395-403
47:481-485
12:715-721
124:375-409
178-186
1338-1343