

Complex I Is Rate-limiting for Oxygen Consumption in the Nerve Terminal*

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Metabolic control analysis was used to determine the spread of control exerted by the electron transport chain complexes over oxygen consumption rates in the nerve terminal. Oxygen consumption rates and electron transport chain complex activities were titrated with appropriate inhibitors to determine the flux control coefficients and the inhibition thresholds in rat brain synaptosomes. The flux control coefficients for complex I, complex II/III, complex III, and complex IV were found to be 0.30 ± 0.07 , 0.20 ± 0.03 , 0.20 ± 0.05 , and 0.08 ± 0.05 , respectively. Inhibition thresholds for complex I, complex II/III, complex III, and complex IV activities were determined to be ~ 10 , ~ 30 , ~ 35 , and $50-65\%$, respectively, before major changes in oxygen consumption rates were observed. These results indicate that, of the electron transport chain components, complex I exerts a high level of control over synaptosomal bioenergetics, suggesting that complex I deficiencies that are present in neurodegenerative disorders, such as Parkinson disease, are sufficient to compromise oxygen consumption in the synaptosomal model of the nerve terminal.

Decreased electron transport chain (ETC)² complex activities in mitochondria have been implicated in the pathogenesis of numerous neurodegenerative disorders, such as Alzheimer disease, Parkinson disease (PD), and Huntington disease (1–6). Complex I (EC 1.6.5.3) activity is decreased by 30–40% in the substantia nigra region of the parkinsonian brain postmortem (4). More recently, it was found that complex I is reduced in the frontal cortex of the PD brain (5). This finding, together with the observations of a mutation in the mitochondrial DNA encoding the ND5 subunit of complex I, has led to suggestions that the complex I decrease may be evident throughout the parkinsonian brain (7, 8). Postmortem studies on the Alzheimer brain found that complex IV (EC 1.9.3.1) activity was reduced by $\sim 27\%$ in the cerebral cortex, by $\sim 37\%$ in the temporal cortex, and by $\sim 52\%$ in the hippocampus (1, 2). Examination of the respiratory chain complexes in the brains of Huntington disease patients showed decreased complex II/III (EC 1.8.3.1) activity in the caudate ($\sim 29\%$) and puta-

men ($\sim 67\%$) and reduced complex IV activity in the putamen ($\sim 62\%$) and caudate nucleus ($\sim 30\%$) in mitochondrial membranes (3, 6).

Using a metabolic control analysis approach, respiratory chain complexes were found to have different flux control coefficient (FCC) values and inhibition threshold levels depending on the tissue source (9, 10). The FCC is a measure of the control exerted by an individual ETC complex over oxidative phosphorylation. Metabolic control analysis studies have been performed on isolated synaptic and nonsynaptic rat brain mitochondria (9–13). However, these studies do not take into account channeling of respiratory substrates to the mitochondria inside a neuron or nerve terminal, which may possibly lead to differences in energy states when compared with *in vivo* mitochondria.

This paper determined the FCCs and inhibition thresholds for complex I, complex II/III, complex III (EC 1.10.2.2), and complex IV over the control of oxygen consumption in *in situ* mitochondria in rat brain synaptosomes.

EXPERIMENTAL PROCEDURES

Materials—Female Wistar rats (~ 250 g) were provided by the Bioresources Unit, Biochemistry Department, Trinity College, Dublin, Ireland. Chemicals were supplied by Sigma or by BDH, Dagenham, Essex, UK.

Preparation of Synaptosomes—The method of synaptosome preparation was based on that by Lai and Clark (14). Two female Wistar rats were killed by cervical dislocation, and the brains were chopped, rinsed, and homogenized using glass Kontes homogenizing tube in STE buffer (320 mM sucrose, 10 mM Tris, 1 mM EDTA, pH 7.4) at 4 °C. The samples were centrifuged at 3,000 rpm for 3 min. Subsequently the supernatants were centrifuged at 10,000 rpm for 10 min. The pellets were resuspended and placed on a discontinuous Ficoll gradient and were centrifuged in an ultracentrifuge at 28,000 rpm for 45 min at 4 °C. Synaptosomes were removed from the Ficoll gradient, were resuspended in STE buffer, and were stored on ice. The protein concentration was calculated using the method of Bradford (15) with bovine serum albumin as the reference standard.

Oxygen Consumption Experiments—The rate of synaptosomal oxygen consumption (JO_2) was determined using a Clark-type oxygen electrode. Synaptosomes (1 mg/ml) were incubated in Krebs buffer (3 mM KCl, 140 mM NaCl, 25 mM Tris-HCl, 10 mM glucose, 2 mM MgCl_2 , 2 mM CaCl_2 , pH 7.4) at 37 °C in the reaction chamber, and the appropriate inhibitor was added, giving a final volume of 3 ml. The inhibitors were

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² The abbreviations used are: ETC, electron transport chain; FCC, flux control coefficient; PD, Parkinson disease.

chosen according to the complex of interest; rotenone was used to inhibit complex I activity, myxothiazol to inhibit complex II/III and complex III activities, and KCN to inhibit complex IV activity. The rates of oxygen consumption were monitored for 6–7 min before the reaction was stopped, and the samples were stored at -80°C for spectrophotometric examination of complex activities.

Electron Transport Chain Assays—The samples prepared from the oxygen consumption experiments were freeze-fractured three times using liquid nitrogen and were examined for their corresponding complex activity. All assays were performed on a Cary UV spectrophotometer at 37°C .

The method used to measure complex I activity was based on a modification of the method of Ragan *et al.* (16), which involved monitoring the oxidation of NADH at 340 nm. 100 μg of synaptosomes were added to plastic cuvettes containing reaction buffer (10 mM MgCl_2 and 25 mM potassium phosphate, pH 7.4) 0.15 mM NADH, 2.5 mg of bovine serum albumin, and 1 mM KCN, giving a final volume of 1 ml. Decylubiquinone (50 μM) was added to start the reaction, and the rates were monitored for 7–8 min. Rotenone (10 μM) was added to obtain the rotenone-insensitive rate for a further 5–6 min.

Complex II/III activity was determined using a modification of the method of King (17), which followed the reduction of cytochrome *c* at 550 nm. Samples (50 μg of synaptosomal protein) were added to plastic cuvettes containing buffer (100 mM potassium phosphate, 0.3 mM potassium-EDTA, pH 7.4), 1 mM KCN, and 100 μM cytochrome *c* with a final volume of 1 ml. The reaction was initiated by the addition of succinate (20 mM). After 7–8 min, myxothiazol (1 μM) was added to determine the myxothiazol-insensitive rate.

Complex III activity was examined using an assay based on the method of Ragan *et al.* (16), which monitored the oxidation of decylubiquinol as an increase in absorbance at 550 nm. Decylubiquinol was prepared from the reduction of decylubiquinone with sodium borohydride and extraction in cyclohexane, dried under nitrogen. The reaction mixture consisted of 25 mM potassium phosphate, 5 mM MgCl_2 , pH 7.2, 2.5 mg/ml bovine serum albumin, 1 mM KCN, 100 μM oxidized cytochrome *c*, 600 μM *n*-dodecyl β -maltoside, 35 μM decylubiquinol, and 10 μM rotenone in 1-ml plastic cuvettes. The reaction was started by the addition of 50 μg of synaptosomal samples, and the rates were followed for 7–8 min. A few grains of ascorbate were added to reduce the remaining cytochrome *c*. The results were expressed as first-order decay rate constants (*k*).

The activity of complex IV was determined using the method of Wharton and Tzagoloff (18), which monitored the oxidation of cytochrome *c* by complex IV as a decrease in absorbance at 550 nm. Cytochrome *c* was reduced with ascorbic acid and passed through a PD₁₀ gel filtration column. The assay cuvettes contained 50 μM reduced cytochrome *c* and 100 μl of potassium phosphate buffer (100 mM), pH 7.0, with a final volume of 1 ml made up with water. The reaction was initiated by the addition of 50 μg of synaptosomes. The results were expressed as first-order decay rate constants (*k*).

Calculation of Flux Control Coefficients—Metabolic control analysis (19, 20) was used to determine the FCCs for the complexes of interest (Equation 1).

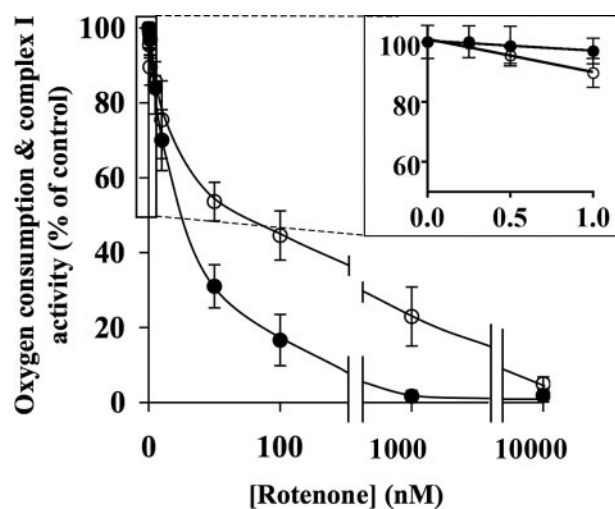


FIGURE 1. Rotenone titration of synaptosomal oxygen consumption and complex I activity. Rat brain synaptosomes were incubated with a series of concentrations of rotenone (0–10 μM) in Krebs buffer at 37°C . The rates of oxygen consumption (●) were measured for 6–7 min. Samples were freeze-fractured, and complex I activity (○) was determined. Oxygen consumption and complex I activity were expressed as percentages of their controls (3.92 ± 0.12 nmol $\text{O}_2/\text{min}/\text{mg}$ and 38.8 ± 4.19 nmol/min/mg, respectively). Experiments were performed on five individual preparations, and results are expressed as mean $\pm$ S.E. (error bars). At points where no error bar is shown, the S.E. was within the size of the symbol. *Inset*, the initial rates of oxygen consumption and complex I activity, between 0 and 1 nM rotenone. The slopes were calculated by linear regression.

$$\text{FCC} = (d\text{JO}_2/d(\text{inhibitor})/d\text{Vc}/d(\text{inhibitor})) \quad (\text{Eq. 1})$$

$d\text{JO}_2/d(\text{inhibitor})$ is the rate of change of oxygen consumption (entire flux) at low concentrations of the inhibitor, and $d\text{Vc}/d(\text{inhibitor})$ is the rate of change of the complex activity at low concentrations of the inhibitor. The lines fitted to the complex activity and oxygen consumption data, at low concentrations of inhibitor, generated r^2 values above 0.9 for the calculations of FCCs.

RESULTS

Titration of Complex I Activity—Increasing concentrations of rotenone decreased the rate of synaptosomal oxygen consumption and the activity of complex I (Fig. 1). Complex I activity decreased linearly to 90% over the rotenone concentration range of 0–1 nM; however, oxygen consumption rates remained between 100 and 97%. As the concentration of rotenone increased above 1 nM, both rates decreased rapidly, with complex I activity falling more quickly than oxygen consumption. The FCC for complex I was calculated by dividing the rate of oxygen consumption, at lower concentrations of rotenone, by the rate of complex I activity, at lower concentrations of the inhibitor (Equation 1), which gave the FCC for complex I as 0.30 ± 0.07 (Table 1). The threshold level of complex I inhibition was determined from a curve that expressed the rate of synaptosomal oxygen consumption as a function of inhibition of complex I activity (Fig. 2). The complex I inhibition threshold was found to be $\sim 10\%$, beyond which the rate of oxygen consumption decreased rapidly. At 40% complex I inhibition, synaptosomal oxygen consumption was reduced by $\sim 60\%$.

Titration of Complex II/III Activity—Myxothiazol decreased the rate of synaptosomal oxygen consumption and the activity

TABLE 1

Flux control coefficient values and inhibition thresholds for complexes I, II/III, III, and IV in rat brain synaptosomes

FCCs and inhibition thresholds were determined using results in Figs. 1–8, as explained under “Experimental Procedures.”

Respiratory chain complex	Flux control coefficient	Inhibition threshold
		%
Complex I	0.30 ± 0.07	~10
Complex II/III	0.20 ± 0.03	~30
Complex III	0.20 ± 0.05	~35
Complex IV	0.08 ± 0.02	50–65

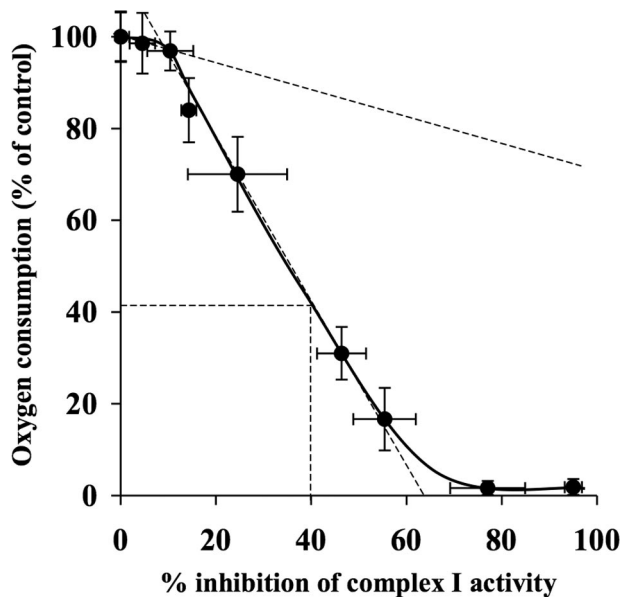


FIGURE 2. Low inhibition threshold for complex I in rat brain synaptosomes. The oxygen consumption results from Fig. 1 were plotted against inhibition of complex I activity (as % of control). Experiments were performed on five individual preparations, and results are expressed as mean $\pm$ S.E. (error bars). At points where no error bar is shown, the S.E. was within the size of the symbol.

of complex II/III in a concentration-dependent manner (Fig. 3). Oxygen consumption rates remained between 100 and 96% activity up to 150 nM myxothiazol, compared with complex II/III activity, which remained between 100 and 79% (Fig. 3). The FCC for complex II/III was calculated as 0.20 ± 0.03 (Table 1). The complex II/III inhibition threshold was determined to be ~30%, beyond which a decline was observed in synaptosomal oxygen consumption rates (Fig. 4).

Titration of Complex III Activity—Up to 150 nM myxothiazol, synaptosomal oxygen consumption remained between 100 and 92% and complex III activity remained between 100 and 63% (Fig. 5), which gave a FCC of 0.20 ± 0.05 (Table 1). The results in Fig. 5 were re-plotted to generate an inhibition threshold curve for complex III (Fig. 6). The complex III inhibition threshold was ~35%.

Titration of Complex IV Activity—As the concentration of KCN increased from 0 to 1 μ M KCN, synaptosomal oxygen consumption decreased from 100 to 96%, whereas complex IV activity was reduced from 100 to 56% (Fig. 7). The FCC for complex IV was calculated to be 0.08 ± 0.02 (Table 1). Using the same sets of results, the complex IV inhibition threshold was determined to be 50–65%, beyond which the rate of synaptosomal oxygen consumption decreased rapidly (Fig. 8).

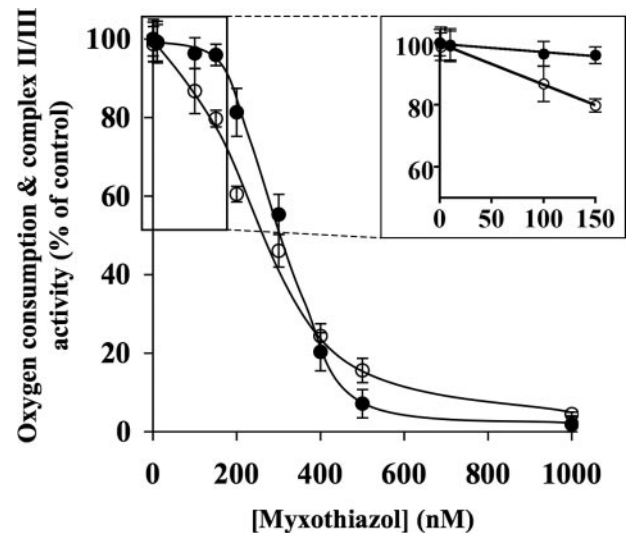


FIGURE 3. Myxothiazol titration of synaptosomal oxygen consumption and complex II/III activity. Rat brain synaptosomes were incubated with a series of concentrations of myxothiazol (0–1 μ M) in Krebs buffer at 37 °C. The rate of oxygen consumption ($\bullet$) was measured for 6–7 min. Samples were freeze-fractured, and complex II/III activity was determined ($\circ$). Oxygen consumption and complex II/III activity were expressed as percentages of their controls (3.92 ± 0.12 nmol $\pm$ O₂/min/mg and 43.2 ± 5.2 nmol/min/mg, respectively). Experiments were performed on five individual preparations, and results are expressed as mean $\pm$ S.E. (error bars). At points where no error bar is shown, the S.E. was within the size of the symbol. *Inset*, the initial rates of oxygen consumption and complex II/III activity, between 0 and 150 nM myxothiazol. The slopes were calculated by linear regression.

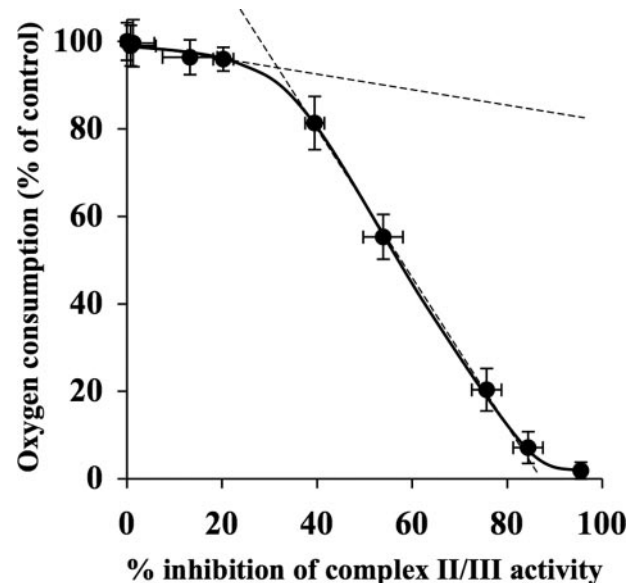


FIGURE 4. Complex II/III inhibition threshold in rat brain synaptosomes. The oxygen consumption results from Fig. 3 were plotted against inhibition of complex II/III activity (as % of control). Experiments were performed on five individual preparations, and results are expressed as mean $\pm$ S.E. (error bars). At points where no error bar is shown, the S.E. was within the size of the symbol.

DISCUSSION

Numerous parameters, such as tissue variation, differing energy states, aging, and varying experimental conditions can lead to contrasting FCC values and inhibition threshold levels (9, 10, 21–23). Analysis of FCCs from different tissue sources found that oxidative phosphorylation in muscle and heart mito-

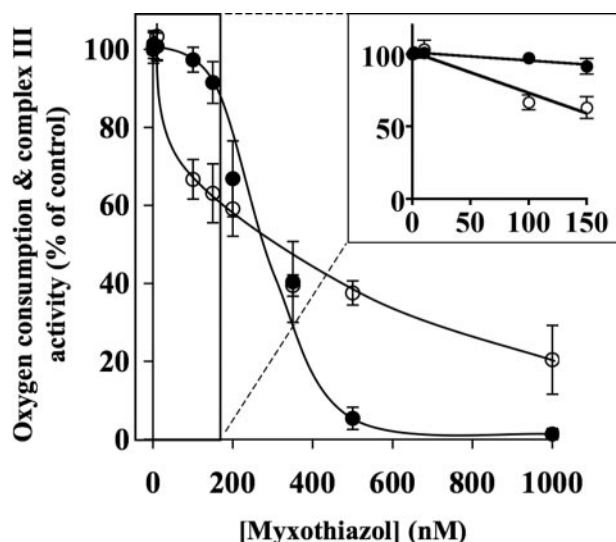


FIGURE 5. **Myxothiazol titration of synaptosomal oxygen consumption and complex III activity.** Rat brain synaptosomes were incubated with a series of concentrations of myxothiazol (0–1 μ M) in Krebs buffer at 37 °C. The rate of oxygen consumption (●) was measured for 6–7 min. Samples were freeze-fractured, and complex III activity was determined (○). Oxygen consumption and complex III activity were expressed as percentages of their controls (3.92 ± 0.12 nmol O_2 /min/mg and 2.72 ± 0.12 k/min/mg, respectively). Experiments were performed on five individual preparations, and results are expressed as mean $\pm$ S.E. (error bars). At points where no error bar is shown, the S.E. was within the size of the symbol. *Inset*, the initial rates of oxygen consumption and complex III activity, between 0 and 150 nM myxothiazol. The slopes were calculated by linear regression.

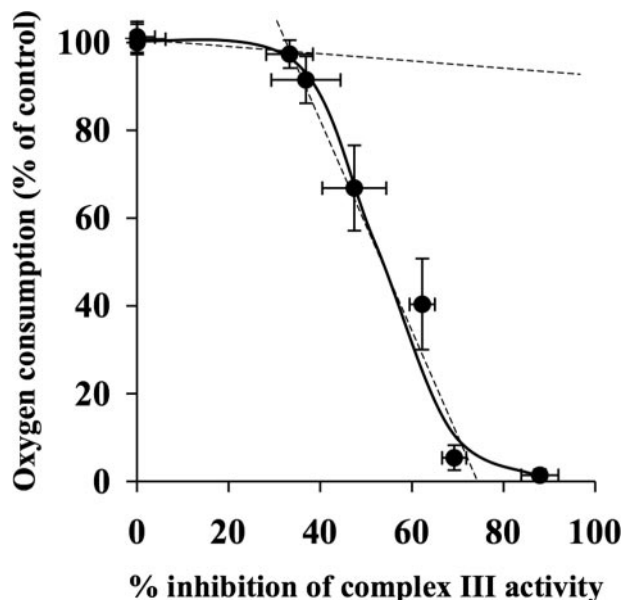


FIGURE 6. **Complex III inhibition threshold in rat brain synaptosomes.** The oxygen consumption results from Fig. 5 were plotted against inhibition of complex III activity (as % of control). Experiments were performed on five individual preparations, and results are expressed as mean $\pm$ S.E. (error bars). At points where no error bar is shown, the S.E. was within the size of the symbol.

chondria was controlled at the level of the respiratory chain, whereas other factors such as ATP synthase and the phosphate carrier play larger roles, along with the respiratory chain complexes, in the control of oxidative phosphorylation in brain, kidney, and liver mitochondria (9). Along with differing tissue sources and varying energy states, age can be an additional fac-

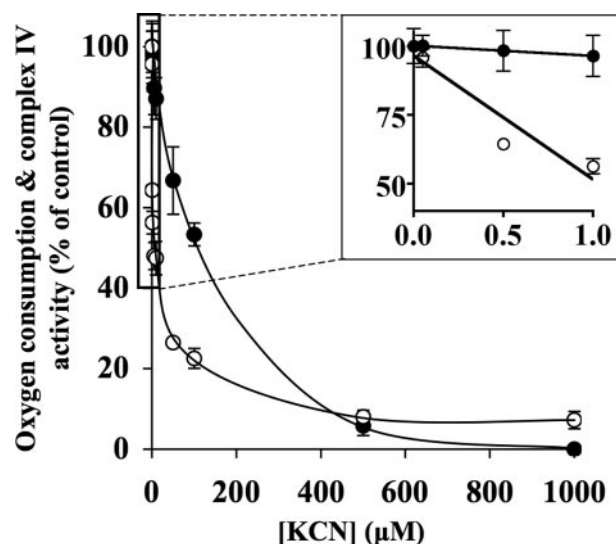


FIGURE 7. **KCN titration of synaptosomal oxygen consumption and complex IV activity.** Rat brain synaptosomes were incubated with a series of concentrations of KCN (0–1 mM) in Krebs buffer at 37 °C. The rate of oxygen consumption (●) was measured for 6–7 min. Samples were freeze-fractured, and complex IV activity was determined (○). Oxygen consumption and complex IV activity were expressed as percentages of their controls (3.92 ± 0.12 nmol O_2 /min/mg and 9.01 ± 0.31 k/min/mg, respectively). Experiments were performed on five individual preparations, and results are expressed as mean $\pm$ S.E. (error bars). At points where no error bar is shown, the S.E. was within the size of the symbol. *Inset*, the initial rates of oxygen consumption and complex IV activity, between 0 and 1 μ M KCN. The slopes were calculated by linear regression.

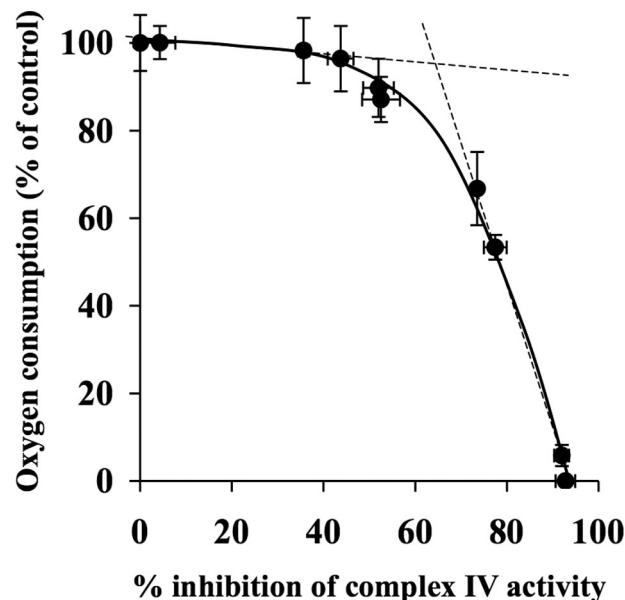


FIGURE 8. **Complex IV inhibition threshold in rat brain synaptosomes.** The oxygen consumption results from Fig. 7 were plotted against inhibition of complex IV activity (as % of control). Experiments were performed on five individual preparations, and results are expressed as mean $\pm$ S.E. (error bars). At points where no error bar is shown, the S.E. was within the size of the symbol.

tor affecting the spread of control. Synaptosomes from young (3 months) and old (24 months) rat brains showed altered patterns of control among substrate oxidation, phosphorylating system, and proton leak in maximally phosphorylating mitochondria (23).

The results in this paper demonstrate that complex I is the major control point of the ETC complexes for oxygen con-

sumption in nerve terminal mitochondria. The FCC value for complex I (0.30 ± 0.07) was higher than those for complex II/III (0.20 ± 0.03), complex III (0.20 ± 0.05), and complex IV (0.08 ± 0.02), and the complex I inhibition threshold level ($\sim 10\%$) was lower than those for complex II/III ($\sim 30\%$), complex III ($\sim 35\%$), and complex IV ($50\text{--}65\%$). Considering the observation that neuronal cells cannot function anaerobically when operating at maximal glycolytic rates (24), impaired activity of the respiratory chain complexes (above the threshold levels) may reduce oxygen consumption in the nerve terminal, with subsequent reductions in ATP (11, 12). Decreased activity of respiratory complexes has been reported in numerous neurodegenerative disorders (1–6). The lower inhibition threshold for complex I implies that complex I defects may have the greatest ability, of the ETC complexes examined, to induce a reduced bioenergetic status in the nerve terminal. A 40% reduction in complex I activity, such as that present in PD, would reduce oxygen consumption in the nerve terminal by $\sim 60\%$.

Metabolic control analysis studies performed on isolated brain mitochondria reported a much lower complex I inhibition threshold for synaptic mitochondria ($\sim 25\%$) than nonsynaptic mitochondria ($\sim 72\%$) (11, 12). In this study, complex I in the synaptosomal mitochondria was inhibited by only $\sim 10\%$ before a decrease in oxygen consumption was observed, which is more in accordance with the level calculated for synaptic mitochondria, as would be expected, because a similar sample of mitochondria exists in isolated synaptic mitochondria preparations as in the synaptosomal preparations. A previous study showed that complex I activity was 36% lower in synaptic mitochondria than in nonsynaptic mitochondria (25), which may somewhat explain the lower thresholds obtained in synaptic and synaptosomal mitochondria when compared with nonsynaptic mitochondria. Similarly, a recent study found that complex I activity in C57BL/6J mouse brain synaptic mitochondria was 45% lower than that in nonsynaptic mitochondria (26).

Above 20% complex I inhibition, the mitochondrial membrane potential decreased significantly in resting rat brain synaptosomes, and above 40% complex I inhibition, glutamate release was increased, whereas ATP levels were reduced in depolarized rat brain synaptosomes (27). Furthermore, $\sim 16\%$ inhibition of complex I activity has been reported to result in increased production of reactive oxygen species in guinea pig synaptosomes (28). Thus, adequate complex I activity appears to be crucial for normal mitochondrial function. Those results, examined along with the results from this study, imply that the complex I inhibition above $\sim 10\%$ could initially cause decreased oxygen consumption, with greater inhibition leading to downstream effects such as mitochondrial membrane potential depolarization, glutamate release, decreased ATP levels, and increased levels of reactive oxygen species.

In this study, the inhibition thresholds for complex II/III and complex III were determined. The complex II/III inhibition threshold was found to be $\sim 30\%$, and the complex III inhibition threshold was calculated to be $\sim 35\%$. The similar inhibition thresholds obtained for complex II/III and complex III imply that complex II does not exert much effect on the complex II/III threshold and that the complex II/III threshold is largely dependent on complex III. In addition,

these results suggest that determination of the inhibition threshold and FCC for complex III can be performed by examining complex II/III activity or complex III activity. The synaptosomal complex III inhibition threshold was lower than the thresholds reported for complex III in nonsynaptic mitochondria ($\sim 70\%$) and synaptic mitochondria ($\sim 80\%$) (10–12).

The complex IV inhibition threshold in *in situ* mitochondria was found to be in the range of $50\text{--}65\%$. The complex IV threshold level was determined to be $\sim 70\%$ in synaptic mitochondria (11) and $\sim 60\%$ in nonsynaptic mitochondria (12), implying that complex IV possesses similar levels of control of oxygen consumption rates in *in situ* mitochondria as in isolated rat brain mitochondria. *In vivo* metabolic control analysis studies, performed on HepG2 cells, showed that complex IV had a lower threshold ($\sim 49\%$) in the absence of oligomycin (state 3 respiration), compared with a threshold of $\sim 75\%$ in the presence of oligomycin (state 4 respiration) (21). Studies have shown that *in situ* synaptosomal mitochondria respire at a rate between state 3 and state 4, with the initial rate being closer to state 4 (29). Therefore, the synaptosomal model is more comparable with the HepG2 cells in the presence of oligomycin.

In accordance with the results reported here, complex I possessed the highest level of control of the ETC complexes in isolated rat brain synaptic mitochondria (11). The FCCs for complex II/III (0.20 ± 0.03) and complex III (0.20 ± 0.05) in synaptosomal mitochondria are more compatible with the FCC for complex III from synaptic mitochondria (0.20) than the FCC from nonsynaptic mitochondria (0.15). The FCC for complex IV in synaptosomes was 0.08 ± 0.02 , implying that the level of control possessed by complex IV in *in situ* mitochondria was lower than that possessed by complex IV in both synaptic and nonsynaptic mitochondria, with FCCs of 0.13 and 0.24, respectively.

Discrepancies between the results for isolated mitochondria and those reported here may be partly accounted for by the observation that isolated mitochondria were provided with a model, such as glutamate and malate or succinate, and the FCCs and inhibition threshold levels were determined with the mitochondria in state 3. This is different from the synaptosomal situation, which involves the generation of endogenous substrates for mitochondria from glucose through the processes of glycolysis and the citric acid cycle.

According to the theory of metabolic control analysis, the sum total of the FCCs of the processes involved in oxidative phosphorylation is equal to 1. Aside from the respiratory chain complexes, other processes that exert control over oxidative phosphorylation are proton leak, phosphate carrier, pyruvate carrier, ATP synthase, adenine nucleotide carrier, glycolysis, and citric acid cycle. It is suggested from our results that the ETC complexes exert a high level of control over oxidative phosphorylation, considering that the FCCs for the ETC complexes account for 0.58 ± 0.12 of the total value.

To conclude, it is evident from the results obtained here that complex I exerted the greatest level of control of the respiratory chain complexes over oxygen consumption in *in situ* mitochondria. Lower inhibition thresholds for complex I, complex II/III, complex III, and complex IV imply that they are more

tightly controlled in *in situ* mitochondria than in the isolated mitochondrial studies. The FCC values for the ETC complexes in synaptosomes show a similar pattern of control over the process of oxygen consumption when compared with that in isolated synaptic mitochondria.

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REFERENCES

1. Kish, S. J., Bergeron, C., Rajput, A., Dozic, S., Mastrogiacomo, F., Chang, L. J., Wilson, J. M., DiStefano, L. M., and Nobrega, J. N. (1992) *J. Neurochem.* **59**, 776–779
2. Maurer, I., Zierz, S., and Moller, H. J. (2000) *Neurobiol. Aging* **21**, 455–462
3. Browne, S. E., Bowling, A. C., MacGarvey, U., Baik, M. J., Berger, S. C., Muqit, M. M., Bird, E. D., and Beal, M. F. (1997) *Ann. Neurol.* **41**, 646–653
4. Schapira, A. H., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., and Marsden, C. D. (1990) *J. Neurochem.* **54**, 823–827
5. Parker, W. D., Jr., Parks, J. K., and Swerdlow, R. H. (2008) *Brain Res.* **1189**, 215–218
6. Brennan, W. A., Jr., Bird, E. D., and Aprille, J. R. (1985) *J. Neurochem.* **44**, 1948–1950
7. Smigrodzki, R., Parks, J., and Parker, W. D. (2004) *Neurobiol. Aging* **25**, 1273–1281
8. Parker, W. D., Jr., and Parks, J. K. (2005) *Biochem. Biophys. Res. Commun.* **326**, 667–669
9. Rossignol, R., Letellier, T., Malgat, M., Rocher, C., and Mazat, J. P. (2000) *Biochem. J.* **347**, 45–53
10. Rossignol, R., Malgat, M., Mazat, J. P., and Letellier, T. M. M. (1999) *J. Biol. Chem.* **274**, 33426–33432
11. Davey, G. P., Peuchen, S., and Clark, J. B. (1998) *J. Biol. Chem.* **273**, 12753–12757
12. Davey, G. P., and Clark, J. B. (1996) *J. Neurochem.* **66**, 1617–1624
13. Davey, G. P., Canevari, L., and Clark, J. B. (1997) *J. Neurochem.* **69**, 2564–2570
14. Lai, J. C., and Clark, J. B. (1976) *Biochem. J.* **154**, 423–432
15. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
16. Ragan, C. I., Wilson, M. T., Darley-Usmar, V. M., and Lowe, P. N. (1987) *Mitochondria, A Practical Approach* (Darley-Usmar, V. M., Rickwood, D., and Wilson, M. T., eds) pp. 79–112, IRL Press at Oxford University Press, Oxford
17. King, T. S. (1967) *Methods Enzymol.* **10**, 217–235
18. Wharton, D. C., and Tzagoloff, A. (1967) *Methods Enzymol.* **10**, 245–250
19. Heinrich, R., and Rapoport, T. A. (1974) *Eur. J. Biochem.* **42**, 89–95
20. Kacser, H., and Burns, J. A. (1973) *Symp. Soc. Exp. Biol.* **27**, 65–104
21. Piccoli, C., Scrima, R., Boffoli, D., and Capitanio, N. (2006) *Biochem. J.* **396**, 573–583
22. Villani, G., and Attardi, G. (2000) *Free Radic. Biol. Med.* **29**, 202–210
23. Joyce, O. J., Farmer, M. K., Tipton, K. F., and Porter, R. K. (2003) *J. Neurochem.* **86**, 1032–1041
24. Pauwels, P. J., Opperdoes, F. R., and Trouet, A. (1985) *J. Neurochem.* **44**, 143–148
25. Almeida, A., Brooks, K. J., Sammut, I., Keelan, J., Davey, G. P., Clark, J. B., and Bates, T. E. (1995) *Dev. Neurosci.* **17**, 212–218
26. Kiebish, M. A., Han, X., Cheng, H., Luncford, A., Clarke, C. F., Moon, H., Chuang, J. H., and Seyfried, T. N. (2008) *J. Neurochem.* **106**, 299–312
27. Kilbride, S. M., Telford, J. E., Tipton, K. F., and Davey, G. P. (2008) *J. Neurochem.* **106**, 826–834
28. Sipos, I., Tretter, L., and Adam-Vizi, V. (2003) *J. Neurochem.* **84**, 112–118
29. Scott, I. D., and Nicholls, D. G. (1980) *Biochem. J.* **186**, 21–33

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