Decylubiquinone Increases Mitochondrial Function in Synaptosomes*

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The effects of decylubiquinone, a ubiquinone analogue, on mitochondrial function and inhibition thresholds of the electron transport chain enzyme complexes in synaptosomes were investigated. Decylubiquinone increased complex I/III and complex II/III activities by 64 and 80%, respectively, and attenuated reductions in oxygen consumption at high concentrations of the complex III inhibitor myxothiazol. During inhibition of complex I, decylubiquinone attenuated reductions in synaptoosomal oxygen respiration rates, as seen in the complex I inhibition threshold. Decylubiquinone increased the inhibition thresholds of complex I/III, complex II/III, and complex III over oxygen consumption in the nerve terminal by 25–50%, when myxothiazol was used to inhibit complex III. These results imply that decylubiquinone increases mitochondrial function in the nerve terminal during complex I or III inhibition. The potential benefits of decylubiquinone in diseases where complex I, I/III, II/III, or III activities are deficient are discussed.

Numerous reports have suggested that ubiquinone (coenzyme Q10) may have beneficial effects in neurodegenerative disorders (1–6). However, it is difficult to utilize ubiquinone in vitro experiments because of its high level of hydrophobicity. Therefore, the beneficial effects of synthesized ubiquinone analogues, such as decylubiquinone (2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone), are under investigation. Decylubiquinone is an exogenous, hydrophobic quinone that has a 10-carbon side chain with a methyl group at the end and can travel into mitochondrial membranes unaided (7). Decylubiquinone accepts electrons from complex I and is reduced to decylubiquinol, which subsequently transfers electrons to complex III. Studies on the effect of decylubiquinone on the steadystate kinetics of complex I in bovine heart mitochondria showed that the binding of decylubiquinone induced a conformational change in the shape of the binding site, which allows the binding of a quinone with a long isoprenoid side chain (8). Decylubiquinone may be used favorably as an alternative to coenzyme Q1 because the interaction of decylubiquinone with complex I is more similar to that between endogenous ubiquinone and complex I than between coenzyme Q1 and complex I and because coenzyme Q1 is not as efficient at activating complex I activity as decylubiquinone (9).

In this study, the effects of decylubiquinone on the activities of a number of electron transport chain (ETC) components, complexes I (EC 1.6.5.3), I/III (EC 1.6.99.3), II/III (EC 1.3.5.1 + 1.10.2.2), and III (EC 1.10.2.2), in rat brain synaptosomes were examined. In addition, the effect of decylubiquinone on synaptoosomal oxygen consumption, during titration with mitochondrial inhibitors, was investigated. Metabolic control analysis may be used to examine the spread of control among components in a system (10, 11), and the inhibition threshold is a useful parameter that describes mitochondrial function. The inhibition threshold is the level by which each component can be inhibited before an effect is observed on the entire flux. In this study, inhibition thresholds were obtained for ETC complexes I, I/III, II/III, and III in the presence and absence of decylubiquinone in rat brain synaptosomes.

EXPERIMENTAL PROCEDURES

Materials—Female Wistar rats (approximately 250 g) were obtained from the Bioresources Unit, Biochemistry Department, Trinity College, Dublin. Chemicals were provided by Sigma or by BDH, Dagenham, Essex, UK. Decylubiquinone was prepared in ethanol.

Preparation of Synaptosomes—The method of Lai and Clark (12) was used to isolate synaptosomes. The preparation of synaptosomes involved the chopping and homogenization of brain samples from two female Wistar rats, followed by centrifugation at 823 g for 3 min at 4 °C. The subsequent supernatants were centrifuged at 9,148 × g for 10 min at 4 °C. The pellets were resuspended and ultracentrifuged on a discontinuous Ficoll gradient at 104,200 × g for 45 min at 4 °C. During the isolation, the samples were maintained in STE buffer (320 mM sucrose, 10 mM Tris, 1 mM EDTA, pH 7.4) and stored on ice. Following extraction of the synaptosomes from the Ficoll gradient, the protein concentration was determined using the method of Bradford (13), with the reference standard of bovine serum albumin.

Oxygen Consumption Experiments—Synaptosomal oxygen consumption rates were investigated with the use of a Clark-type oxygen electrode. Krebs buffer (3 mM KCl, 140 mM NaCl, 25 mM Tris-HCl, 10 mM glucose, 2 mM MgCl2, 2 mM CaCl2, pH 7.4) was used as the reaction buffer for the experiments. Synaptosomes (1 mg/ml) were incubated in Krebs buffer at 37 °C in the oxygen electrode. Decylubiquinone or ethanol and the

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The abbreviations used are: ETC, electron transport chain; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like syndrome; MERRF, myoclonic epilepsy associated with ragged-red fibers.
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**RESULTS**

Effects of Decylubiquinone on ETC Complex Activities in Synaptosomes—Decylubiquinone (50 μM) significantly increased complex I/III activity by 64%, from 51.7 ± 5.7 nmol/min/mg in the control to 85 ± 8.3 nmol/min/mg (Fig. 1). Complex II/III activity was increased by the addition of 50 μM decylubiquinone from 33.8 ± 6.8 nmol/min/mg in the control to 61 ± 5.7 nmol/min/mg in the decylubiquinone-treated sample, an increase of 80% (Fig. 1).

Effect of Decylubiquinone on Synaptosomal Oxygen Consumption during Titrations with Mitochondrial Inhibitors—No significant difference was observed between the rate of synaptosomal oxygen consumption in the control in the absence of 50 μM decylubiquinone compared with that in the presence of 50 μM decylubiquinone (Figs. 2–4). Decylubiquinone did not alter oxygen consumption in synaptosomes during...
inhibition with the complex I inhibitor, rotenone (Fig. 2). However, decylubiquinone significantly attenuated reductions in oxygen consumption at higher concentrations of myxothiazol (350 nM and 500 nM; Fig. 3) and at higher concentrations of antimycin A (50 nM, 100 nM, and 500 nM; Fig. 4) compared with samples that had not been treated with decylubiquinone.

**Effect of Decylubiquinone on Inhibition Thresholds for Complex I**—Rotenone titrations of oxygen consumption rates and complex I activity were used to determine the inhibition thresholds. Fig. 5 shows the inhibition thresholds for complex I in the absence and presence of 50 μM decylubiquinone. The approximate inhibition threshold in the absence of decylubiquinone was found to be 10% compared with that of...
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Decylubiquinone does not alter complex I/III inhibition threshold in synaptosomes when titrated with rotenone. Rat brain synaptosomes were incubated with a series of concentrations of rotenone (0–10 μM) in the absence and presence of 50 μM decylubiquinone in Krebs buffer in an oxygen electrode at 37 °C. Rates of oxygen consumption and corresponding complex I/III activity were determined and expressed as percentages of their controls and used to generate the complex I/III inhibition threshold curves in the absence (●) and presence (○) of decylubiquinone. The control rates of complex I/III activity in the absence and presence of decylubiquinone were 53 ± 6.6 nmol/min/mg and 79.5 ± 9.2 nmol/min/mg, respectively. Experiments were performed on five individual preparations, and results are expressed as mean ± S.E. (error bars).

Effect of Decylubiquinone on Inhibition Thresholds for Complex I/III—Both rotenone and myxothiazol titrations of oxygen consumption rates and complex I/III activity were used to determine the inhibition thresholds. The complex I/III inhibition threshold, found by titrating with rotenone, showed a similar pattern in the absence and presence of 50 μM decylubiquinone (Fig. 6). However, the curve for decylubiquinone shifted to the right compared with the control curve. The complex I/III inhibition threshold curve in the absence of decylubiquinone was 10%, and that for the decylubiquinone-treated samples was 15% (Table 1). At 42% inhibition of complex I/III, synaptosomal oxygen consumption rates were 57% of the control rate in the presence of decylubiquinone, in contrast to 21% in the absence of decylubiquinone.

Effect of Decylubiquinone on Inhibition Thresholds for Complexes II/III and III—Fig. 7 shows that the inhibition threshold for complex II/III in the absence and presence of decylubiquinone was 41%, in contrast to 21% in the absence of decylubiquinone. As expected, the presence of decylubiquinone increases the inhibition threshold for complex II/III in the absence of decylubiquinone.

Inhibition thresholds for complexes I, I/III, II/III, and III in the absence and presence of decylubiquinone

TABLE 1

|                      | −Decylubiquinone | +Decylubiquinone |
|----------------------|------------------|------------------|
| Complex I (rotenone) | 10               | 15               |
| Complex I/III (rotenone) | 10             | 15               |
| Complex I/III (myxothiazol) | 35           | 85               |
| Complex II/III (myxothiazol) | 40           | 90               |
| Complex III (myxothiazol) | 35           | 60               |

Decylubiquinone increases complex II/III inhibition threshold in synaptosomes, when titrated with myxothiazol. Rat brain synaptosomes were incubated with a series of concentrations of myxothiazol (0–1 μM) in the absence and presence of 50 μM decylubiquinone in Krebs buffer in an oxygen electrode at 37 °C. Rates of oxygen consumption and corresponding complex II/III activity were determined and expressed as percentages of their controls and used to generate the complex II/III inhibition threshold curves in the absence (●) and presence (○) of decylubiquinone. The control rates of complex II/III activity in the absence and presence of decylubiquinone were 42.2 ± 4.8 nmol/min/mg and 73.8 ± 8.25 nmol/min/mg, respectively. Experiments were performed on five individual preparations, and results are expressed as mean ± S.E. (error bars).

Effect of Decylubiquinone on Inhibition Thresholds for Complex II/III—Fig. 8 shows that the inhibition threshold for complex II/III in the absence of decylubiquinone was 40%. However, the presence of decylubiquinone appeared to increase the threshold to 90% (Table 1).
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The inhibition thresholds provide information on the level by which the activity of a complex can be inhibited before detrimental effects are seen on synaptosomal oxygen consumption. We showed previously that complex I can be inhibited up to 10% before major effects on synaptosomal oxygen consumption are observed (22). This is a low threshold showing that the activity of complex I is very important in maintaining adequate synaptosomal oxygen consumption. In the current study, a low complex I threshold of 15% was reported in the presence of decylubiquinone. The complex I inhibition threshold appears to decrease more slowly in the presence of decylubiquinone than in its absence, which suggests that decylubiquinone may have the ability to attenuate reduction in oxygen consumption rates associated with reduced complex I activity. Complex I activity is reduced by 30–40% in the substantia nigra and frontal cortex in Parkinson’s disease (23, 24). In the present study, at 40% inhibition of complex I activity, ~40% synaptosomal oxygen consumption remained in the absence of decylubiquinone, in contrast to ~70% in the presence of decylubiquinone, suggesting that decylubiquinone may have a protective effect in this situation.

When myxothiazol was used to inhibit complex III, the inhibition thresholds for complexes I/III, II/III, and III in the presence of decylubiquinone were all higher than those without decylubiquinone, which may be of interest when considering treatments for disorders involving complex III deficiencies. This observation may be explained by the apparent protective effect of decylubiquinone on synaptosomal oxygen consumption at high concentrations of myxothiazol. Therefore, higher levels of complex III inhibition were required to bring about deleterious effects on synaptosomal oxygen consumption. Investigation of this effect with an alternative complex III inhibitor, antimycin A, reported results similar to those with myxothiazol. Decylubiquinone maintained synaptosomal oxygen consumption rates in the presence of high concentrations of antimycin A. Considering the observation that myxothiazol and antimycin A inhibit at alternative sites in the Q cycle in complex III (25), the site of inhibition at complex III was irrelevant with regard to the effects of decylubiquinone on oxygen consumption rates.

Decylubiquinone remains largely in its reduced form, decylubiquinol, in rat liver mitochondria that are respiring on succinate (26). It may be the case that decylubiquinol can interfere with myxothiazol partitioning into complex III, thereby affecting the inhibition of complex III. This would attenuate the inhibition of oxygen consumption at higher concentrations of myxothiazol. In this case, decylubiquinol would also lessen the reduction of complex III activity by myxothiazol; however,
Decylubiquinone did not affect complex I/III, complex II/III, or complex III activities at higher concentrations of myxothiazol. This implies that myxothiazol is gaining access to complex III to the same extent in the absence and in the presence of decylubiquinone. If decylubiquinone is not protecting oxygen consumption through decylubiquinol competition with myxothiazol, it may be the case that decylubiquinone enhances oxygen consumption by increasing electron flow through the ETC, which brings about elevated rates of oxygen reduction at complex IV. Decylubiquinone may maintain the flux of electrons from complex III to cytochrome c, which could increase the rate of oxygen consumption, even in the presence of myxothiazol, because myxothiazol has its actions before the transfer of electrons from complex III to cytochrome c.

Complex I/III (titrated with myxothiazol) and complex II/III inhibition thresholds appear to switch from type II threshold curves, in the absence of decylubiquinone, to type I threshold curves in the presence of decylubiquinone. A type I curve is characterized by a plateau phase which, at a point, decreases sharply and rapidly allowing the threshold to be precisely calculated (27). A type II threshold is defined as a curve that does not fall as steeply or quickly as a type I curve, making the determination of the threshold less straightforward (27). Switching between type II and type I curves was observed previously when the substrate available to rat liver mitochondria was changed from succinate to pyruvate (27). Alteration between type II and type I curves can occur as a result of changes in the activity of the enzyme so that it becomes available in excess or so that its activity is up-regulated, accounting for the plateau phase. In this study, it could be the case that decylubiquinone increases the substrate available to complex III, thereby sustaining complex III activity for longer than in the absence of decylubiquinone.

Inhibition thresholds can be used to postulate the effects of a drug on mitochondrial function. From the results in this study, it appears that decylubiquinone may have advantageous effects in diseases in which deficiencies in complexes I, I/III, I/II, and III have been reported. Decylubiquinone altered the appearance of the complex I threshold so that synaptosomal oxygen consumption did not fall as rapidly as in the absence of decylubiquinone. Therefore, decylubiquinone may have beneficial effects on oxygen consumption in the nerve terminal at certain levels of complex I inhibition as seen in Parkinson disease. The pathogenesis of certain mitochondrial diseases, such as Leber hereditary optic neuropathy (LHON), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome (MELAS), and myoclonic epilepsy associated with ragged-red fibers (MERRF) has been shown to involve mutations in genes that encode for certain ETC complex subunits, in particular the subunits of complex I (28–30). A number of mutations have been shown to occur in LHON, with one mutation in particular, G3460A, associated with reduced complex I activity of 60–80% (31, 32). Complex I activity was reduced in fibroblasts from MERRF and MELAS patients by 24 and 78%, respectively (33). The results in the present study suggest that decylubiquinone may have beneficial effects in these diseases by attenuating oxygen consumption rates during complex I inhibition. We have shown previously that low concentrations of the complex I inhibitor, rotenone, brought about depolarization of mitochondrial membrane potential, reduced ATP levels and increased glutamate release from depolarized rat brain synaptosomes (34), which may be ameliorated by the addition of decylubiquinone.

There are numerous reports of alterations in respiratory chain complex activities with aging. The activity of complex I has been reported as being lower in aged rat brain mitochondria (35), complex I/III activity was reduced by 48% in brain mitochondria of aged mice (36), and the activities of complexes II and III were significantly lower in aged mouse brain mitochondria than in young samples (37). Previously, we have demonstrated the occurrence of an age-related decrease in complex II/III activity in in situ rat brain nerve terminal mitochondria (38). In the present study, decylubiquinone increased the inhibition thresholds for complexes I/III, II/III, and III, suggesting that decylubiquinone may induce advantageous effects in age-related diseases and in disorders involving deficiencies of these ETC enzymes, by maintaining sufficient oxygen consumption at higher levels of complex III inhibition than in the absence of decylubiquinone treatment.

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