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Mechanism of inhibition of mitochondrial respiratory complex I by 6-hydroxydopamine and its prevention by desferrioxamine

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Abstract

Inhibition of mitochondrial complex I by 6-hydroxydopamine was studied in brain and liver preparations. NADH–quinone reductase activity of this complex from rat brain was inhibited by 6-hydroxydopamine partially uncompetitively with respect to NADH with a value of K_i 0.051 ± 0.014 mM. The inhibition patterns for liver NADH–quinone reductase were more complicated than those obtained with the brain enzyme. Desferrioxamine behaved as a 'competitive' activator of complex I from both liver and brain (K_a = 2 mM and 0.02 mM, respectively). It also protected brain complex I against the inhibition by increasing K_i value about 10-fold. Furthermore, in the presence of desferrioxamine the residual activity of enzyme–substrate–inhibitor complex was increased. The data suggest that desferrioxamine does not compete directly with 6-hydroxydopamine for binding to the inhibitory site, but induces a conformation which is unfavorable for the binding of the inhibitor to the protein. The qualitative and quantitative differences between the behavior of the liver and brain enzyme complexes indicate that the assumption that the behavior of liver mitochondria can be used as a model for the situation in brain should be reconsidered.

Keywords: Mitochondrial respiratory complex I; 6-Hydroxydopamine; Inhibition; Kinetic mechanism; Protection by desferrioxamine; Kinetic mechanism

1. Introduction

The neurotoxicity of catecholamines was shown to be associated to their inhibition of the enzymes of mitochondrial respiratory chain, especially of complex I (NADH dehydrogenase) (Ben-Shachar et al., 1991; Glinka and Youdim, 1995). Our earlier results (Glinka et al., 1996) demonstrated that the inhibition of rat brain NADH dehydrogenase (EC 1.6.99.3) by 6-hydroxydopamine is completely reversible and there is no significant involvement of free radicals in the process. Iron(III) did not enhance the susceptibility of the enzyme to inhibition. In apparent contrast to the above data, although in a good agreement with those for dopamine inhibition (Ben-Shachar et al., 1994, 1991) desferrioxamine was able to protect the enzyme activity against inhibition by 6-hydroxydopamine. However, other chelating agents and antioxidants did not protect the enzyme suggesting that another property of desferrioxamine might underlie its ability to protect the enzyme from inhibition. The following study is devoted to the investigation of the protective ability of desferrioxamine and to the kinetic mechanism of the inhibition of complex I by 6-hydroxydopamine.

2. Materials and methods

2.1. Mitochondria isolation

Male Sprague–Dawley rats 300–450 g were killed by decapitation. The brains were immediately extracted and cooled in the ice-cold isotonic 10 mM Tris–HCl buffer, pH 7.5, containing 0.25 M sucrose, and 2% bovine serum albumin (free of fatty acids). Intact mitochondria were isolated from the brain by differential centrifugation with recentrifugation in 16% iso-osmotic percoll for the final purification (Gavin et al., 1990). The preparation contained approximately 25–35 mg of protein ml^{-1}, and aliquots were either used immediately or kept frozen at −20°C for up to 2 weeks. Each aliquot that was thawed was used only once. This storage process caused no loss of the enzyme

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activity or sensitivity to inhibition. Rat liver mitochondria were isolated according to the same procedure. The purity of the mitochondrial preparation was estimated indirectly using the sensitivity of NADH–quinone reductase to inhibition by rotenone (see below under Section 2.2). The percoll purification step increased this index from 70 to 95% approximately, indicating that any NADH dehydrogenases not related to coenzyme Q were essentially completely removed from the preparation. Protein concentration was measured using Biuret reaction in modification of King (1967).

2.2. Determination of NADH dehydrogenase activity

Mitochondrial suspensions were diluted with 10 mM Tris–HCl buffer, pH 7.4, containing 0.25 M sucrose. The final protein concentration in the assay mixture was 0.07 mg ml^{-1} for brain and 0.01 mg ml^{-1} for liver mitochondria for all assays. The buffer system used was 50 mM Tris–HCl, pH 7.4. All measurements were at 25°C. Each assay aliquot of mitochondrial suspension was permeabilized for 1 min with 1% digitonin, added directly to the mitochondrial suspension in the reaction cuvette before mixing with buffer. This process caused complete access of all substrates to their binding sites. This was confirmed by the comparable behavior of the NADH dehydrogenase activities in digitonin-treated and sonicated mitochondria. NADH dehydrogenase activity was measured in an assay system, containing varied NADH concentrations, 0.1 mM decylubiquinone, a synthetic water-soluble analog of ubiquinone (coenzyme Q), and 3 mM potassium cyanide (this reaction is further referred to as NADH–quinone reductase). The reaction was started by addition of decylubiquinone and NADH oxidation was monitored by following the decrease in optical density of NADH at 340 nm (Singer, 1974). This reaction was 95% inhibited by 0.5 μM rotenone. Addition of 0.5 μM antimycin did not affect significantly the kinetic behavior of NADH–quinone reductase and it was omitted from the assay system in subsequent studies.

For the NADH–ferricyanide reductase assay decylubiquinone was replaced by the alternative electron acceptor 0.1 mM potassium ferricyanide. This concentration was found to be saturating. Potassium cyanide was omitted, since preliminary experiments demonstrated no effect of this respiratory inhibitor on the kinetic behavior. Addition of 0.5 μM rotenone also revealed no influence on the inhibition of NADH–ferricyanide reductase by 6-hydroxydopamine, and it was omitted from the routine assay procedure. Since non-enzymatic oxidation of 6-hydroxydopamine under some experimental conditions may produce significant amounts of colored semi-quinone, the assays were run routinely in parallel with blanks, in which NADH was omitted, and the final results were corrected for the blank values. Comparison of the changes in absorbance at 420 nm and 340 nm in the ferricyanide reductase assay showed an exact correlation between NADH decline and ferricyanide reduction. This confirms that, under the conditions used, ferricyanide interacts predominantly with the mitochondrial respiratory chain and not with the other components of the assay system, such as 6-hydroxydopamine.

In all cases the time of preincubation of the assay mixture containing permeabilized mitochondria, buffer, 6-hydroxydopamine and other components, excluding the substrates, was strictly 1 min from mixing to the start of the reaction.

2.3. Reagents

All the reagents were of the highest purity that could be purchased from Sigma. Desferrioxamine was a gift from Ciba Geigy (Switzerland). 6-Hydroxydopamine solutions in 2 mM dithionite were prepared freshly each day and stored on ice. Lower dithionite concentrations were found to be ineffective in preventing the oxidative loss of the 6-hydroxydopamine whereas higher concentrations interfered with the assay. The final dithionite concentration added with 6-hydroxydopamine to the reaction mixture was in the range 2–10 μM.

2.4. Statistics

For plotting data and regression analyses the computer program Inplot 4 was used.

3. Results

3.1. Prevention of 6-hydroxydopamine oxidation

We have previously demonstrated the inhibition of mitochondrial respiration by 6-hydroxydopamine by a process that did not involve iron or free radicals (Glinka and Youdim, 1995; Glinka et al., 1996). However, no detailed analysis of the mechanism of that inhibition was presented.

There was no significant difference between freshly-prepared and frozen mitochondria which had been thawed but not refrozen. Therefore mitochondrial suspensions that had been stored frozen were used throughout this work. Digitonin-permeabilized mitochondria were freely permeable to the substrates, as demonstrated by comparison of the kinetic curves obtained with digitonin-treated and sonicated mitochondria (results not shown).

6-Hydroxydopamine is readily oxidizable in both monoamine oxidase and non-enzymatic reactions (see the work of Glinka and Youdim (1995)). To examine whether this might affect the accuracy of the inhibition studies through inhibitor depletion, the assay mixture containing 6-hydroxydopamine was incubated in the presence and absence of the non-selective monoamine oxidase inhibitor tranylcypromine and varied concentrations of antioxidant dithionite before assay (Fig. 1). Since there was no differ-

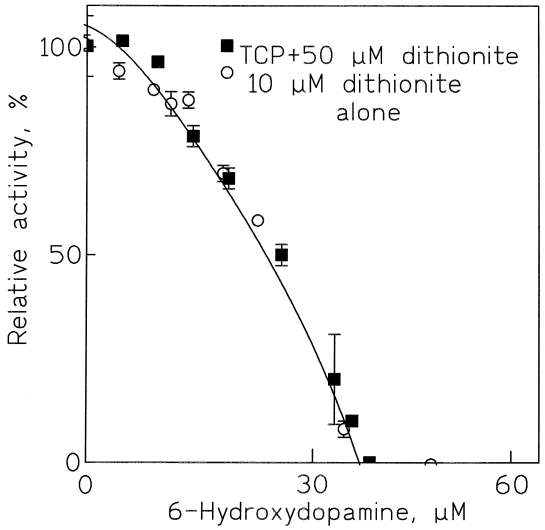


Fig. 1. Mitochondrial suspension from rat brain was preincubated with non-selective inhibitor of monoamine oxidase tranylcypromine (1 μM) for 20 min and then antioxidant (50 μM dithionite) and the other components of the assay mixture for ferricyanide reductase assay were added according to the procedure described in the text. This curve was plotted together with another one, obtained without tranylcypromine and with 10 μM dithionite.

ence between the curves it can be concluded that there was no significant oxidation of 6-hydroxydopamine by monoamine oxidase under the condition used here, and that 10 μM dithionite was as effective as a 50 μM concentration of this antioxidant in protecting against loss of 6-hydroxydopamine by non-enzymatic oxidation. All the following data were obtained using low dithionite concentration and in the absence of inhibitors of monoamine oxidase. High concentrations of dithionite can cause redox recycling of 6-hydroxydopamine/ferricyanide or 6-hydroxydopamine/decylubiquinone pairs resulting in artifacts in the kinetic curves (data not shown). The chosen dithionite concentration did not produce detectable oxidative-reductive recycling of ferricyanide and decylubiquinone, but still protected the inhibitor from oxidative loss. Throughout this work, saturating concentrations of the electron acceptors were used, since lower concentrations might be significantly reduced by the 6-hydroxydopamine/dithionite pair. Therefore, the interaction of 6-hydroxydopamine with complex I at constant NADH and varied ferricyanide or decylubiquinone concentrations could not be investigated. It is very unlikely that use of an alternative antioxidant would exclude the above interactions. No interaction between NADH and the 6-hydroxydopamine/dithionite pair was detected.

Table 1
Rat liver and brain NADH–ferricyanide reductases and NADH—quinone reductases inhibition by 6-hydroxydopamine

NADH (μM)	NADH–ferricyanide reductase		NADH–quinone reductase	
	n_H^a	IC_{50}^b (μM)	n_H^a	IC_{50}^b (μM)
Brain				
12	1	17	–	–
24	1.4	22	1	500
48	2.2	20	1.6	580
78	2.8	17	1.5	650
120	3.5	18	2	600
240	2.3	19	–	–
Liver				
6	1.0	39	–	–
12	2.9	25	–	–
24	7.3	24	2.6	380
240	2.6	24	3.8	354

[a] n_H represents the Hill constant.
[b] IC_{50} is the inhibitor concentration that gives rise to 50% inhibition under the experimental conditions.

Table 2
The influence of 6-hydroxydopamine on V_{max} and K_m for brain and liver complex I

6-hydroxydopamine (μM)	V_{max} (mmol min^{-1} mg protein^{-1})	±S.E.M.	K_m (μM)	±S.E.M.
Brain NADH–quinone reductase				
0	0.062	0.007	11.8	0.8
235	0.056	0.002	3.6	0.08
275	0.046	0.007	2.7	0.9
295	0.043	0.002	1.7	0.9
Liver NADH–quinone reductase				
0	0.146	0.009	21.78	0.62
118	0.070	0.002	43.67	0.36
235	0.045	0.004	84.75	0.032
353	0.0011	0.001	30.30	0.072

3.2. Kinetic analysis of the inhibition of brain and liver NADH dehydrogenase activities by 6-hydroxydopamine

To estimate the selectivity of inhibition, kinetic data for liver and brain mitochondrial complex I were compared. Previous work (Glinka et al., 1996) had demonstrated inhibition of the brain enzyme to be reversible. The specific NADH–ferricyanide reductase activities were 1.75 ± 0.08 μmol min^{-1} mg of protein^{-1} and 0.164 ± 0.004 μmol min^{-1} mg^{-1} for liver and brain enzyme, respectively, and the NADH–quinone reductase activities were 0.146 ± 0.009 and 0.063 ± 0.007 mmol min^{-1} mg^{-1} of protein, respectively. The above differences in the specific activities of quinone and ferricyanide reductases are similar to that published before (Hatefi et al., 1979). The liver and brain enzymes were inhibited by 6-hydroxydopamine both in the quinone reductase and ferricyanide reductase reactions in an apparently cooperative process with Hill coefficients that increased with increasing NADH concentration but with IC_{50} value being unaffected (Table 1). Ferricyanide reductase activities demonstrated higher apparent cooperativity than quinone reductase activities.

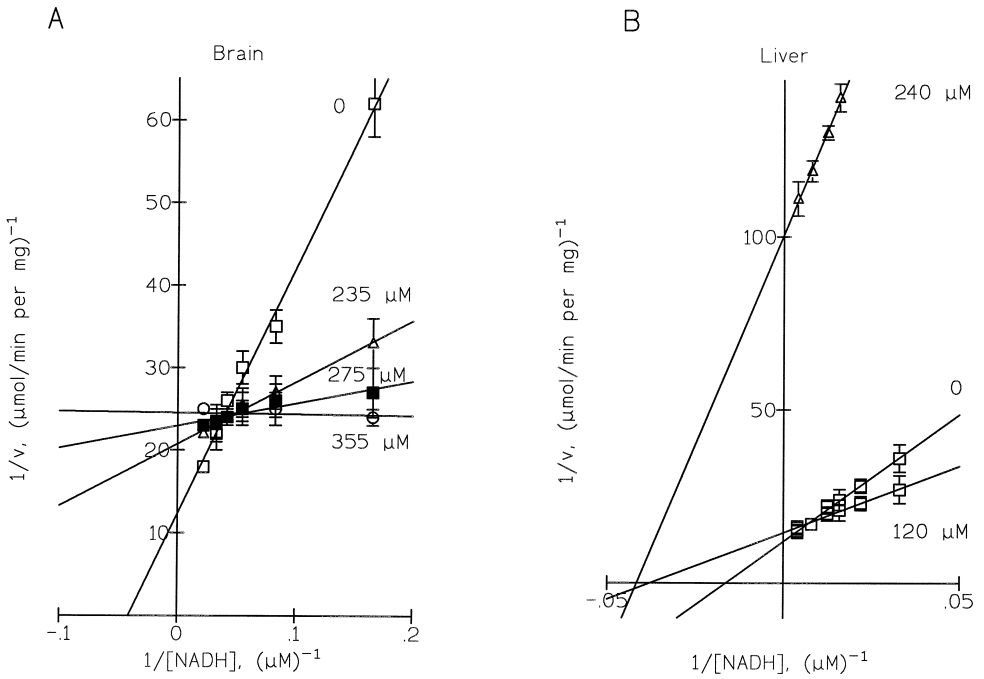
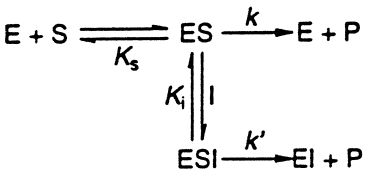


Fig. 2. Double-reciprocal plots for brain and liver NADH–quinone reductase with increasing 6-hydroxydopamine concentrations.

Although the NADH dehydrogenase reaction involves two substrates, at saturating concentrations of quinone and ferricyanide it may be treated as a pseudo-monosubstrate reaction. The variation of initial velocity with NADH concentration for quinone reductases from both sources followed simple Michaelis–Menten kinetics (data not shown). In agreement with earlier findings (Singer, 1974), both ferricyanide reductases were inhibited by the highest

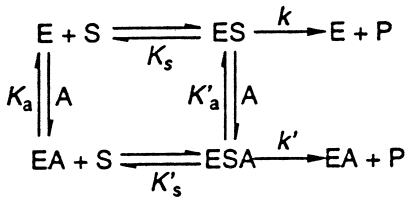
(a) partially uncompetitive inhibition by 6-hydroxydopamine.



$$v = \frac{V}{1 + \frac{K_s}{s} + \frac{i}{K_i}} + \frac{V'}{1 + \frac{K_i}{i}\left(1 + \frac{K_s}{s}\right)}$$

$$= \frac{V}{\frac{K_S}{\left(1 + \frac{(V'/V)i}{K_i}\right)s}} + \frac{1 + i/K_i}{1 + \frac{(V'/V)i}{K_i}}$$

(b) competitive non-essential activation by desferrioxamine



$$v = \frac{V}{1 + \frac{K_s}{s}\left(1 + \frac{a}{K_a}\right) + \frac{a}{K'_a}} + \frac{V'}{1 + \frac{K'_a}{a} + \frac{K'_s}{s} + \frac{K_s K'_a}{a s}}$$

$$= \frac{V\frac{s}{K_s} + V'\frac{a s}{K'_a K_s}}{1 + \frac{s}{K_s} + \frac{a}{K_a} + \frac{a s}{K'_a K_s}}$$

Scheme 1. Possible interactions of 6-hydroxydopamine and desferrioxamine with rat brain mitochondrial complex I. (a) Partially uncompetitive inhibition by 6-hydroxydopamine. (b) Competitive non-essential activation by desferrioxamine. In both cases e, s, i and a represent concentrations of enzyme, substrate, inhibitor and activator concentrations; K_s, K_i etc. represent dissociation constants for the designated reactions and: $V = ke$ and $V' = k'e$.

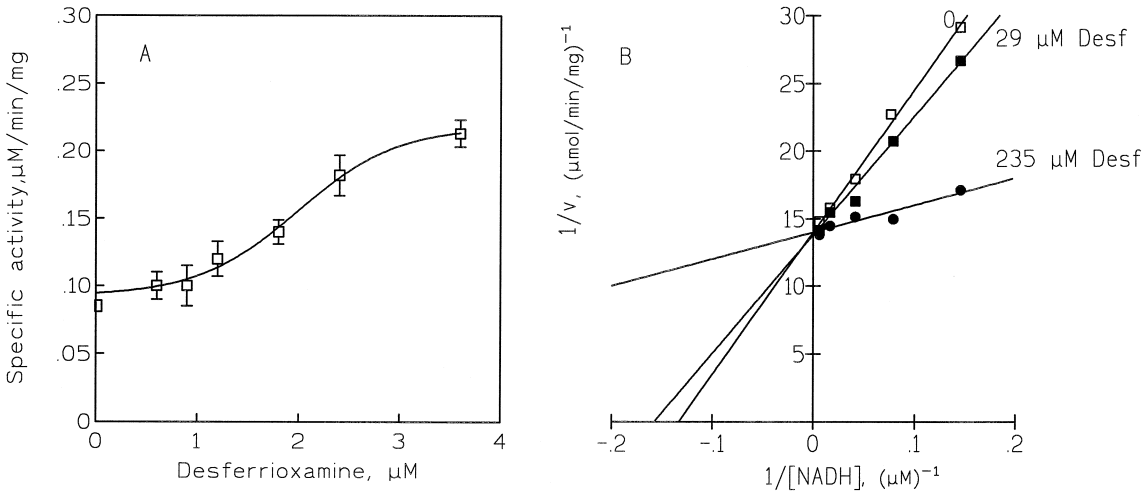


Fig. 3. Activation of brain NADH–quinone reductase by desferrioxamine in the presence of 120 μM NADH (A) and double-reciprocal plots with increasing desferrioxamine concentrations (B).

of NADH concentrations tested (240 μM), but in a range from 6 to 120 μM the dependence also followed Michaelis–Menten equation. 6-Hydroxydopamine strongly inhibited both liver and brain enzyme with respect to NADH in a process that affected both K_m value and the maximum velocity (Table 2). The mode of inhibition of ferricyanide reductase activity was complicated with no common intersection point of the double reciprocal plot lines at different inhibitor concentrations being obtained (data not shown). There was however no evidence of a competitive model with any tested concentration range of 6-hydroxydopamine. Unlike ferricyanide reductase activities, brain quinone reductase was inhibited in an apparently partially uncompetitive way by 6-hydroxydopamine (Fig. 2a and Scheme 1a) with an inhibitor constant of 0.051 ± 0.014 mM, calculated by regression analysis of the data. The inhibition pattern for liver quinone reductase was more complicated, thus precluding simple analysis of inhibitor type and calculation of the inhibitor constant (Fig. 2b).

3.3. Activation of brain and liver NADH–quinone reductase by desferrioxamine

Desferrioxamine interferes with ferricyanide reductase assay system in the presence of 6-hydroxydopamine, therefore it was tested only in quinone reductase reaction. Desferrioxamine activated both liver and brain enzymes, although brain enzyme was much more sensitive to the activation by low concentrations of this compound, than

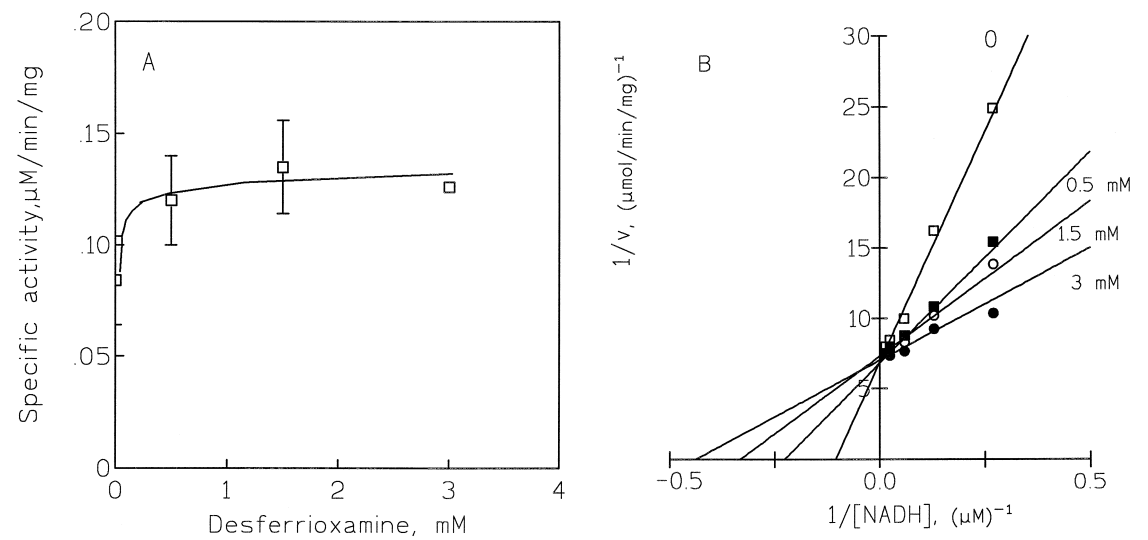


Fig. 4. Activation of liver NADH–quinone reductase by desferrioxamine in the presence of 120 μM NADH (A) and double-reciprocal plots with increasing desferrioxamine concentrations (B).

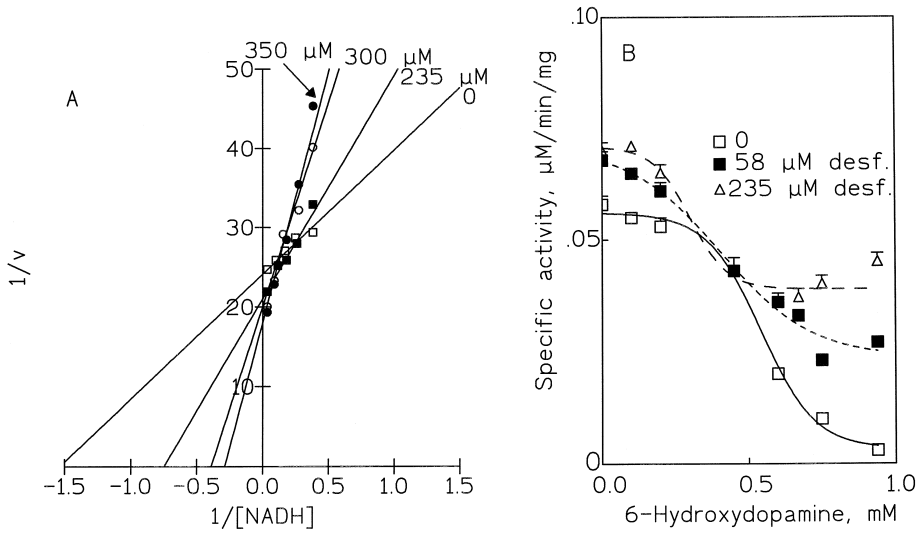


Fig. 5. Protection of brain NADH–quinone reductase by desferrioxamine against inhibition by 6-hydroxydopamine. (A) Double-reciprocal plots in presence of 2 μM desferrioxamine and indicated micromolar concentrations of 6-hydroxydopamine; (B) Inhibition by 6-hydroxydopamine in the absence and presence of 58 and 235 μM desferrioxamine.

that from liver. It activated both enzymes in a 'competitive' manner (Figs. 3 and 4). Desferrioxamine may be regarded as non-essential activator. Hence for the calculation of the activator constant (K_a) a model appropriate for non-essential competitive activation (see the work of Dixon and Webb (1979)) was used. The activator constants determined in this way were 20 μM and 2 mM for brain and liver, respectively.

3.4. Protection of NADH–quinone reductase activities by desferrioxamine

Desferrioxamine was found to protect liver complex I against its inhibition by 6-hydroxydopamine as had been shown earlier for the brain enzyme (Glinka et al., 1996): 90% inhibition by 0.47 mM 6-hydroxydopamine was completely prevented by 0.3 mM desferrioxamine. The more detailed kinetic analysis shows this protection to be complex. K_i for inhibition of brain NADH–quinone reductase by 6-hydroxydopamine increased from 0.05 ± 0.01 mM to 0.43 ± 0.02 mM in the presence of 2 μM desferrioxamine (see Fig. 5). However, the partial nature of the inhibition by 6-hydroxydopamine was greatly accentuated. As shown in Fig. 5b any residual activity at very high 6-hydroxydopamine concentration in the absence of desferrioxamine was so low that it was not statistically significant. However, it increased considerably with the increasing desferrioxamine concentration. The residual activity estimated as V'/V (see Scheme 1) increased in presence of 2 μM desferrioxamine from 0.1 to 0.9.

4. Discussion

Although the different specific activities of liver and brain complex I (see Table 2) might simply be a result of different degrees of purity of the two preparations, the quantitative differences in their responses to reversible inhibition by 6-hydroxydopamine and activation by desferrioxamine suggest that there are fundamental differences in the catalytic and regulatory behavior of the two enzyme systems. In case of reversible inhibition, sensitivities towards inhibitors would not be expected to be influenced by the concentration of the enzyme, unless the inhibitor (or activator) constant is of the same order or lower than that of the enzyme. The values reported here show that this cannot be the case. Thus this conclusion has an important bearing on the frequently-made assumption that the behavior of liver mitochondria represents an adequate model for the situation in the brain. A tissue difference of the magnitude revealed in the present studies suggests that detailed comparison of the functions of mitochondrial components obtained from the synaptosomal and extra-synaptosomal compartments of brain might be a valuable topic for future work. The brain mitochondrial preparation would have contained extrasynaptosomal and synaptosomal mitochondria, both of which would be rendered accessible by detergent treatment.

The observation that inhibition of complex I functions by 6-hydroxydopamine shows high degrees of apparent cooperativity (see Table 1) would suggest that multiple binding sites and, perhaps, different conformational states are involved in the inhibitory process. Consistent with such an interpretation is the large difference in the apparent cooperativity and inhibitor sensitivity of the NADH–quinone and NADH–ferricyanide reductase activities. Thus occupation of different binding sites by the inhibitor exerts different controlling effects on the sequences of electron transfer steps that occur in this multicomponent system. The existence of these distinct sites at which ubiquinones can interact as electron acceptors with complex I has

recently been proposed (Degli Esposti et al., 1996). The greater complexity and sensitivity to inhibition of ferricyanide reductase activity might arise from this acceptor interacting with the cytochrome *c* and complex III in addition to complex I under the conditions used or with the inhibitor interacting at a site not involved in quinone reduction (see the work of Wyatt et al. (1995)).

The absence of any significant effect on the IC_{50} value for 6-hydroxydopamine inhibition when the NADH concentration was varied would indicate that inhibition is not competitive towards the donor substrate and thus involves the inhibitor binding to the sites other than occupied by NADH. This is supported by the failure to observe competitive behavior in the kinetic analysis of the effects of this inhibitor in either the quinone or ferricyanide reductase assay. Unfortunately, as discussed previously (see Section 3), it was not possible to vary the concentration of the acceptor substrate (either decylubiquinone or ferricyanide) because of unfavorable redox potential relative to the 6-hydroxydopamine/dithionite pair. The possibility of developing a less-readily oxidizable analogue to this compound for investigating this aspect of the inhibitory process might be profitable line for further study.

Perhaps because of the lower cooperativity of the inhibition of the quinone reductase activity by 6-hydroxydopamine as compared to that of ferricyanide reductase, the kinetic behavior of the inhibitory process for the brain system appears to be less complicated. Indeed the kinetic patterns observed in Fig. 2 are consistent with inhibition following a partially uncompetitive pattern, in which the inhibitor binds only to the enzyme–substrate complex, and the resulting ternary enzyme–substrate–inhibitor complex possesses a reduced but finite catalytic activity. In this relatively unusual system, a compound will behave as an inhibitor at higher substrate concentrations but as an apparent activator at lower substrate concentrations. Such behavior of the brain enzyme with respect to NADH concentrations is shown in Fig. 6, where apparently normal Michaelis–Menten kinetic behavior in the presence of a moderate 6-hydroxydopamine concentration is converted to one showing partial high-substrate inhibition by its high concentration. Such kinetic mechanism and the kinetic equation that defines the process (see the work of Tipton (1995)) is shown in Scheme 1a.

Although this mechanism appears to provide an adequate description of the inhibitory behavior and allowed the calculation of K_i values, it is of course possible to devise more complicated kinetic schemes that would also be consistent with such behavior. Indeed, if it is assumed that steady-state conditions, rather than those of thermodynamic equilibrium apply, the resultant kinetic equation becomes more complex than that shown in Scheme 1a and may give rise to higher order kinetic behavior. Since there are multiple sites of interaction of ubiquinone with complex I (Degli Esposti et al., 1996), the possibility that the inhibitor does not interact with each of them might be considered as an explanation. Furthermore as discussed above, the possibility of deviations from this simple behavior cannot be excluded if the inhibitor could be used in even wider range of concentrations. Indeed, the more complex behavior of the liver system in this respect might support such a possibility unless it is taken as a reflection of the fundamental differences in the behavior of complex I in the two tissues.

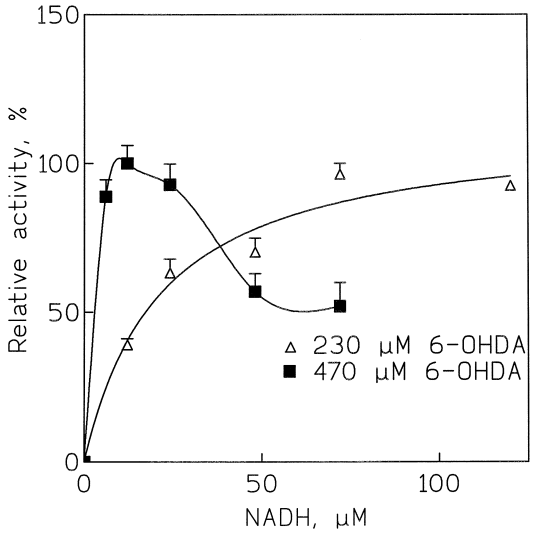


Fig. 6. Induction of substrate inhibition of brain NADH–quinone reductase by high concentration of 6-hydroxydopamine (470 μM). The data is plotted as relative activity, where maximal achieved activity for each curve was considered as 100%. Lower concentration of 6-hydroxydopamine did not induce substrate inhibition.

Unfortunately, as discussed earlier, technical problems precluded studies on the effect of desferrioxamine on the inhibition of the NADH–ferricyanide reductase activity so that this effect could be only investigated for quinone reductase activity. Desferrioxamine was found to behave as a non-essential 'competitive' activator of the enzyme complex. Such behavior can be most simply explained by the binding of the activator to the enzyme increasing its affinity for substrate as shown in Scheme 1b (see the work of Dixon and Webb (1979)). Although there are many examples where small molecules, such as metal ions, have been observed to act in this way through binding to the active site of the enzyme, the relative size of desferrioxamine might make such a direct effect unlikely, and the possibility of this compound interacting at a site, or sites, distinct from the NADH-binding site and exerting its effects through conformational changes might be considered more probable. The observation that the non-essential activation process obeyed simple Michaelis–Menten kinetics does not exclude the possibility of there being multiple binding sites for the activator, provided that such sites are independent and non-interacting. The 100-fold difference between the K_a values for activation of the brain and liver enzymes provides further support for the view that attempts to interpret the behavior of liver mitochondria as a

model for those in brain should be regarded with extreme caution.

The ability of desferrioxamine to protect against inhibition by 6-hydroxydopamine has been shown not to be a result of either metal-chelating or its antioxidant properties (Glinka et al., 1996). Although the ability to activate the enzyme complex might be expected to give rise to an apparent protection, where the inhibitory effect of 6-hydroxydopamine was, to some extent, masked by an independent activation by desferrioxamine, this cannot provide an adequate explanation for the protection kinetics observed. Such a mechanism would simply result in the activity being increased by a constant factor in the presence of a fixed desferrioxamine concentration and not the complex behavior observed. Direct competition for the same binding site(s) as an explanation for the protection is also not adequate, since 6-hydroxydopamine behaves as a partially uncompetitive inhibitor whereas desferrioxamine is a 'competitive' non-essential activator. Some interaction between the binding sites for these two compounds is indicated by the increase of K_i values for 6-hydroxydopamine inhibition that occurs in the presence of desferrioxamine, however this is accompanied by a large increase in the residual activity of the enzyme–substrate–inhibitor complex (V'/V increasing from 0.1 to 0.9, see Fig. 5b and also Scheme 1). The possibility that desferrioxamine stimulates one of the two alternative pathways that can lead to ubiquinone reduction might be investigated by studying the effect of a series of quinone analogues since they have been shown to interact to different extents with the sites involved (Degli Esposti et al., 1996).

Since the selective dopamine neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) exerts its effects, through its metabolite MPP + (1-methyl-4-phenylpyridinium), by direct inhibition of mitochondrial respiration at the level of complex I [see the work of Tipton and Singer (1993)], a process that has itself been reported to result in generation of oxygen radicals [see the works of Hasegawa et al. (1990) and Cleeter et al. (1992)], it is tempting to assume that the catecholaminergic neurotoxin 6-hydroxydopamine acts in a similar way. The present work indicates that the inhibitory potency of this compound is higher than that of MPP + or the isoquinoline derivatives which have been proposed to be endogenous neurotoxins [see the works of Mizuno et al. (1989) and Suzuki et al. (1992)]. Although 6-hydroxydopamine can act as a generator of free radicals, it appears that its direct inhibitory effects on mitochondrial complex I activity does not involve this property (Glinka et al., 1996). This does not, of course, exclude the involvement of oxidative mechanisms in damage to other cellular functions by this compound or the possibility that longer-term exposure of mitochondria to 6-hydroxydopamine might lead to irreversible effects from radicals resulting from inhibition of the respiratory chain or from the catechol derivative itself. The protection afforded by desferrioxamine has been frequently used as an argument to support the view that iron and/or oxidative mechanisms play a central role in the mechanisms of neurodegeneration induced by many toxins. Although this may indeed be the case, the observation that this compound can protect against the inhibitory effects of 6-hydroxydopamine by a mechanism that appears to be independent of its metal chelating or antioxidant properties indicates that such simple interpretations are unjustified and that additional evidence would be required to establish the involvement of oxidative and iron-dependent mechanisms.

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