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Flunarizine and Cinnarizine Inhibit Mitochondrial Complexes I and II: Possible Implication for Parkinsonism

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SUMMARY

Cinnarizine and flunarizine are piperazine derivatives with calcium antagonist and anticonvulsant properties and are used widely in the treatment of vertigo and circulatory disorders. They have been implicated recently in the aggravation, or even the induction, of parkinsonism in elderly patients. Because the aetiology of parkinsonism has been suggested as having a mitochondrial component, we have investigated the effects of both compounds on mitochondrial respiration and on the activities of the individual respiratory chain complexes. In intact mitochondria from rat liver, both drugs inhibited respiration rates, with substrates entering at Complex I (glutamate/malate) and Complex II (succinate). These effects could be explained by potent inhibitions (K_i 3–10 μM) of both complexes. Complex I is inhibited at a site near the ubiquinone-binding site, which is not competitive with respect to ubiquinone, whereas the inhibition of Complex II is apparently caused by competition with ubiquinone. Furthermore, the inhibition of NADH oxidation by flunarizine in submitochondrial particles caused an NADH-dependent generation of superoxide. These inhibitory properties of both compounds could be significant factors in the aggravation or induction of parkinsonism in elderly patients, in whom mitochondrial function already may be impaired.

Cinnarizine is a selective calcium antagonist with anticonvulsant and antihistaminic properties, and is used widely in the treatment of vertigo and peripheral circulatory disorders. The difluorinated derivative, flunarizine, has an enhanced potency and longer circulatory half-life, and it is especially effective in cerebrovascular areas (1). Neither compound was known to have any major side effects, but an increasing number of reports have associated their use with the aggravation, or even the induction, of parkinsonism in elderly patients (2–5).

Parkinsonism has been associated with mitochondrial dysfunction. The evidence relies on the demonstration of deficiencies in activity and content of subunits of Complex I (NADH-ubiquinone reductase) of the mitochondrial respiratory chain in Parkinson's disease patients (6–10). This has been suggested to be caused by the deletion of mitochondrial DNA (11, 12), although other groups have reported that Parkinson's patients do not display any significant increase over the age-related phenomenon of mtDNA deletions (10, 13). Furthermore, parkinsonism may be induced in animals and man by the administration of a mitochondrial toxin, MPTP. The toxicity of this compound is dependent on its oxidation by monoamine oxidase B in the astrocytes to produce a toxic metabolite, MPP^+, which is an inhibitor of Complex I of the respiratory chain (14). MPP^+ is transported actively into the mitochondrial matrix, where its concentration can be as large as 20 mM (14). At this concentration, MPP^+ inhibits respiration and, hence, destroys the affected cell. The selective action of MPTP *in vivo* on the substantia nigra relies on the specific uptake of MPP^+ into the nigrostriatal cells by the synaptic dopamine transport system (15).

We have investigated the effects of flunarizine and cinnarizine on mitochondrial function *in vitro* using the models with which MPTP toxicity has been established, i.e., intact rat liver mitochondria and heart submitochondrial particles. Our results show that both compounds inhibit Complexes I and II of the respiratory chain. These effects offer an explanation for the induction of parkinsonism by these drugs.

Materials and Methods

Ubiquinone$_{-1}$ was a gift of the Eisai Co., Ltd. (Tokyo, Japan). Ubiquinone$_{-2}$ was a gift of Takeda Chemical Industries, Ltd. (Osaka, Japan). Flunarizine and cinnarizine were from Janssen Research (Beerse, Belgium). The sources of other biochemicals were given previously (16, 17). Stock solutions of the drugs in ethanol were prepared daily, and serial dilutions were prepared and contained the same final

ABBREVIATIONS: cinnarizine, [(E)-1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)-piperazine]; BSA, bovine serum albumin; DCPIP, 2,6-dichlorophenolindophenol; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; MPP^+, 1-methyl-4-phenylpyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; RCR, respiratory control ratio; TMPD, tetramethyl-*p*-phenyldiamine; UQ_n, ubiquinone with *n* isoprene units; flunarizine, [(E)-1-(bis)-(4-fluorophenylmethyl)-4-(3-phenyl-2-propenyl)-piperazine]; SMP, submitochondrial particles.

concentration of ethanol (≤1% v/v) in each assay; controls were assayed in the presence of the same concentration of ethanol.

Rat heart (16) and liver (17) mitochondrial fractions were prepared as described. SMP were prepared essentially as in Sanadi *et al.* (18). Briefly, rat heart mitochondria stored at −20° were defrosted and diluted to 5 mg of protein/ml in 250 mM sucrose/10 mM Tris (pH 7.4) containing 1 mM EDTA. After sonication (3 × 30 sec, 50 W, Branson sonifier with microtip), unbroken mitochondria were removed by centrifugation (12,000 × *g* for 10 min). SMP were sedimented from the supernatant at 105,000 × *g* for 45 min, were washed once in sucrose/Tris, and were stored at −80° at 10 mg/ml in the same buffer. Proteins were determined by the method of Lowry (19) with BSA as a standard. Oxygen consumption was assayed polarographically with either 10 mM succinate, 10 mM glutamate, and 1 mM malate, or 10 mM ascorbate and 0.25 mM TMPD as substrates (17). State 3 oxidation was initiated by the addition of ADP (0.33 μM final concentration in the assay) after 3 min of preincubation with the drugs. State 3, state 4, RCR, and ADP:O were measured according to Estabrook (20).

Respiratory chain activities were assayed at 30° in 1 ml of a standard buffer (35 mM KH_2PO_4, 5 mM $MgCl_2$, 2 mM KCN, pH 7.2) (16), with additions as noted below. NADH-cytochrome *c* reductase was measured by the increase in absorbance at 550 nm of 50 μM cytochrome *c* with freeze-thawed mitochondria in the presence of 100 μM NADH. For succinate-cytochrome *c* reductase, the enzyme, 1 μg/ml of rotenone, 0 to 100 μM UQ_{-1}, and 25 mM succinate were preincubated at 30° for 10 min before starting the reaction by the addition of cytochrome *c* (50 μM final concentration). Complex I was assayed at 340 nm as the rotenone-sensitive oxidation of 130 μM NADH with 8 to 100 μM UQ_{-1} as the final electron acceptor. The values of K_m and V_{max} were calculated by nonlinear least squares fitting to a hyperbola using a computer program (Ultrafit, Biosoft, Cambridge, England). NADH-dependent ferricyanide reduction was assayed at 420 nm with 0.5 mM $K_3(FeCN)_6$, 1 μg/ml of rotenone, and 0.5 mM NADH. Complex II was measured at 600 nm as the rate of reduction of 88 μM DCPIP in the presence of 1 μg/ml of rotenone, 1 μg/ml of antimycin A, and 25 mM succinate. The enzyme was incubated in the assay mixture for 10 min, and the reaction was started by the addition of UQ_{-1} to reach a 0 to 100 μM final concentration. Complex III was assayed at 550 nm as the antimycin-sensitive reduction of 25 μM cytochrome *c* with 40 μM ubiquinol$_{-2}$ in the presence of 0.5 mM EDTA. Ubiquinol$_{-2}$ was prepared freshly from UQ_{-2} as described (16).

Superoxide generation was monitored as the rate of adrenochrome formation from 1 mM adrenaline in a dual-wavelength spectrophotometer (Aminco DW-2) using 485/575 nm as the wavelength pair (21), with 100 μg of SMP in 250 mM Sucrose/10 mM Tris, pH 7.2 and 100 μM NADH as substrate. In preliminary experiments, we noted that the lag phase associated with this assay method (21) could be eliminated by the inclusion of adrenochrome, which appeared to accelerate the autocatalytic process (22). For this reason we routinely added to the assay cuvette 10 μM adrenochrome, prepared by leaving a well aerated solution of 1 mM adrenaline in sucrose-Tris at 4° for several days. Blank rates, assayed in the absence of NADH, were subtracted from the results. The addition of 25 units of superoxide dismutase caused an inhibition of more than 95% of the activity. One unit of enzyme activity corresponds to the formation of 1 μmol of product/minute in the defined conditions.

Results

Overall effects of cinnarizine and flunarizine on both the respiration rates and oxidative phosphorylation, with glutamate/malate and succinate as substrates, were studied with intact rat liver mitochondria. The results in Fig. 1 show that the state-3 respiration rates with both substrates were inhibited with either flunarizine or cinnarizine. Flunarizine appeared to be more potent than cinnarizine, because 10 μM flunarizine and 100 μM cinnarizine had about the same inhibitory effect. Fig. 1 also shows that, with succinate, the inhibition was the same whether mitochondria were coupled or uncoupled by the addition of 1 μM FCCP. The concentrations of flunarizine (10 μM) and cinnarizine (100 μM) that caused approximately 30% inhibition of state 3 oxidation had no significant effects on the efficiency of oxidative phosphorylation (ADP:O ratio) or on the respiratory control ratio, an index of the coupling of respiration to ATP synthesis, with succinate as a substrate (Table 1). However, with glutamate/malate, both compounds significantly decreased the ADP:O ratio. With these substrates the RCR

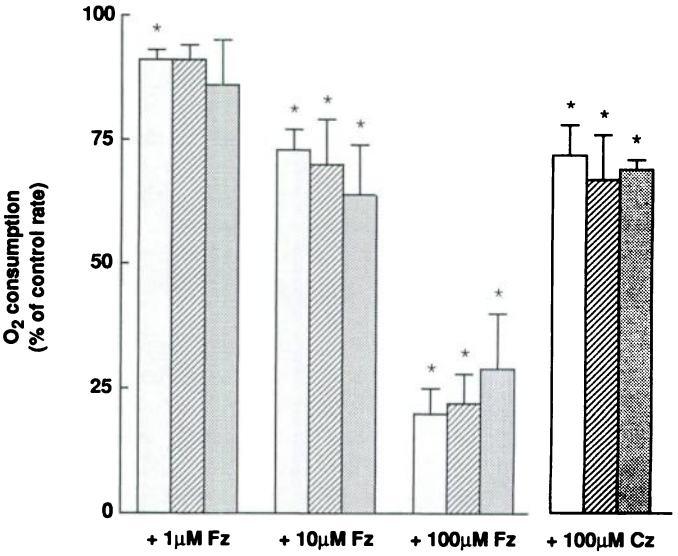

Fig. 1. Inhibition of state 3 oxidation rates by flunarizine and cinnarizine. State 3 oxidation rates of rat liver mitochondria were measured in the presence of 1, 10, and 100 μM flunarizine or 100 μM cinnarizine. Control state 3 rates for 10 mM glutamate + 1 mM malate (*open columns*) and 10 mM succinate (*hatched columns*) were 105 and 149 ng of atoms O · min^{-1} · (mg of protein)$^{-1}$, respectively in coupled mitochondria. The control rate with 10 mM succinate in mitochondria uncoupled by the addition of 1 μM FCCP (*dotted columns*) was 192 ng of atoms O · min^{-1} · (mg of protein)$^{-1}$. The values are the means (+standard error bars) of three to four different preparations (*$p < 0.05$ versus control).

TABLE 1

Respiratory activities in rat liver mitochondria with 10 μM flunarizine and 100 μM cinnarizine

Rates were measured polarographically in intact mitochondria with 10 mM succinate (and 2.5 μM rotenone), 10 mM glutamate, and 1 mM malate, or 10 mM ascorbate and 0.25 mM TMPD as substrates. NADH oxidation was measured in mitochondria disrupted by freeze-thawing and sonication using 0.25 mM NADH as substrate, with 10 μM cytochrome *c* added. Coupled state 3 rates (+ADP), state 4 rates, uncoupled state 3 rates (+1 μM FCCP), and NADH oxidation are expressed as ng of atoms O · min^{-1} · (mg of protein)$^{-1}$. The values are means (±standard error) for three to five different preparations. * $p < 0.05$ versus control values without addition of drugs.

Substrate	No addition	+10 μM Flunarizine	+100 μM Cinnarizine
Succinate			
State 3	149 ± 9	102 ± 9*	95 ± 11*
State 4	29 ± 7	26 ± 3	25 ± 4
RCR	6.5 ± 1.6	4.3 ± 0.4	4.1 ± 0.9
ADP:O	2.02 ± 0.04	1.95 ± 0.06	1.90 ± 0.12
Uncoupled State 3	192 ± 13	126 ± 12*	131 ± 4*
Glutamate/malate			
State 3	105 ± 7	79 ± 8*	72 ± 8*
State 4	11 ± 2	15 ± 2	16 ± 3
RCR	10.4 ± 1.4	6.0 ± 0.9*	5.0 ± 1.2*
ADP:O	2.95 ± 0.07	2.69 ± 0.09*	2.72 ± 0.11*
Ascorbate/TMPD			
State 3	151 ± 8	161 ± 6	156 ± 9
NADH oxidation	181 ± 11	136 ± 4*	123 ± 6*

also was decreased because the inhibition of state 3 was not associated with any decrease in state 4 oxidation; rather, the latter displayed a small nonsignificant increase.

When respiration rates were measured with NADH in liver mitochondria disrupted by sonication, the inhibition was the same as in intact mitochondria with glutamate/malate (Table 1), indicating that the effect was directly on the respiratory chain, rather than on substrate transport. Because ascorbate oxidation with TMPD was not affected by either drug (Table 1), the inhibition must occur before cytochrome *c* and Complex IV.

Classically, the effects of compounds such as MPTP on the activities of the various respiratory chain complexes have been studied using SMP prepared from beef heart mitochondria (14). We preferred to use rat heart mitochondria as these were prepared routinely from fresh tissue in this laboratory in the course of other investigations. We first verified that the inhibition of flunarizine applied not only to liver mitochondria, but also to disrupted rat heart mitochondria by measuring the rates of cytochrome *c* reduction with either NADH (Complexes I and III) or succinate (Complexes II and III) as substrates. Mitochondria were disrupted by three cycles of freeze-thawing (−20/+20°). As shown in Fig. 2, flunarizine markedly inhibited both activities, with 50% inhibition in the 1 to 10 μM range. Furthermore, we found that rat brain mitochondria displayed the same sensitivity to these inhibitions (not shown), showing that these effects are not tissue-specific.

Rat heart SMP then were used to study the inhibitory effect of flunarizine on the four electron-transferring complexes of the respiratory chain. Complex I (NADH-ubiquinone reductase) activity was assayed by the rotenone-sensitive rate of NADH oxidation with 100 μM UQ_{-1} as final electron acceptor. Flunarizine displayed a similar pattern of inhibition on this activity (50% inhibition at 1–10 μM) (Fig. 3) as against NADH-cytochrome *c* reductase (Fig. 2), but there was no significant effect against NADH-ferricyande reductase activity (Fig. 3). This inhibition of Complex I was a result of a decrease in the V_{max} rather than from competition with respect to $Ubiquinone_{-1}$ (Fig. 4). The apparent K_m (40 μM) was not affected by 3 or 10 μM flunarizine (both 39 μM), whereas V_{max} was decreased from 2.91 to 2.22 and 1.21 μmol of NADH · min^{-1} · (mg of protein)$^{-1}$, respectively. This is compatible with a binding of the inhibitor at, or near to, the rotenone-binding site. This effect is similar to that of MPP^+ (23).

The assays of NADH-cytochrome *c* reductase and Complex I described above were performed with 1 mg/ml of defatted BSA in the medium. The latter increased the observed Complex I activity by about 45%, but was without effect on the degree of inhibition by flunarizine. In contrast, cinnarizine was also a potent inhibitor of Complex I, but this inhibition could be largely prevented by the inclusion of BSA in the assay medium. Thus, 10 μM cinnarizine caused a 43% inhibition of Complex I in the absence of BSA, but this inhibition was decreased to 14% in the presence of 1 mg/ml of BSA. There was no effect of 1 mg/ml of γ-globulin on the Complex I activity, or on the inhibitions by flunarizine and cinnarizine.

To analyze the inhibition of succinate-cytochrome *c* reductase activity (Fig. 2), Complex II activity in SMP was assayed as the succinate-dependent reduction of DCPIP. To reveal the full potential activity of Complex II, 100 μM $ubiquinone_{-1}$ was included routinely as an intermediate electron acceptor in this assay (16). There was no effect of either 100 μM flunarizine or 100 μM cinnarizine (not shown) on this activity in the presence of 100 μM UQ_{-1}. However, in the absence of exogenous UQ_{-1}, there was a substantially lower rate, which was inhibited by flunarizine (Table 2). Furthermore, the inhibition of succinate-cytochrome *c* reductase by 10 μM flunarizine and 10 μM cinnarizine could be relieved almost completely by addition of UQ_{-1} (Fig. 5, Table 2). This suggests that the observed inhibitions of succinate oxidation in intact liver mitochondria and of succinate-cytochrome *c* reductase in disrupted heart and brain mitochondria were caused by inhibition of Complex II activity caused by competition between the piperazines and the endogenous ubiquinone.

The activity of Complex III, assayed as the antimycin-sensitive reduction of cytochrome *c* by $ubiquinol_{-2}$, was increased, rather than inhibited, by both drugs. The control rate of 2.27 μmol of cytochrome *c* · min^{-1} · (mg of protein)$^{-1}$ was increased

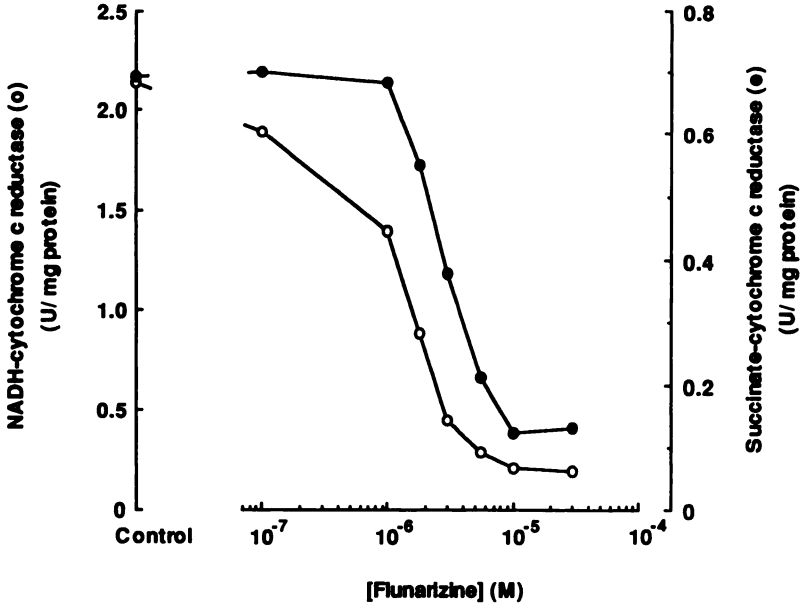

Fig. 2. Inhibition by flunarizine of cytochrome *c* reduction with 100 μM NADH (O) or 25 mM succinate (●) as substrates with freeze-thawed mitochondria from rat heart. Assays were performed in triplicate with 1% (vM) ethanol in the assay cuvette.

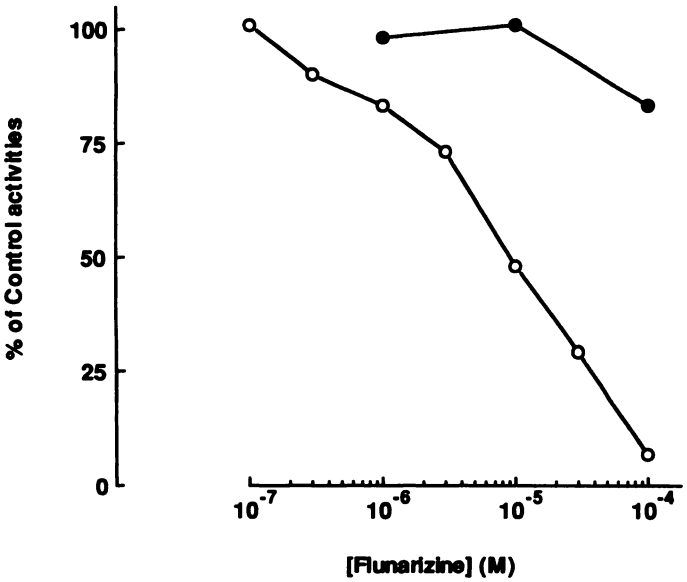

Fig. 3. Inhibition of Complex I activity by flunarizine. The effects of flunarizine on NADH-ubiquinone$_{-1}$ reductase (O) was assayed in the presence of 1 mg/ml of BSA [control rate = 1.73 μmol of NADH · min^{-1} · (mg of protein)$^{-1}$], and on NADH-ferricyanide reductase (●) [control rate = 5.8 μmol of FeCN · min^{-1} · (mg of protein)$^{-1}$].

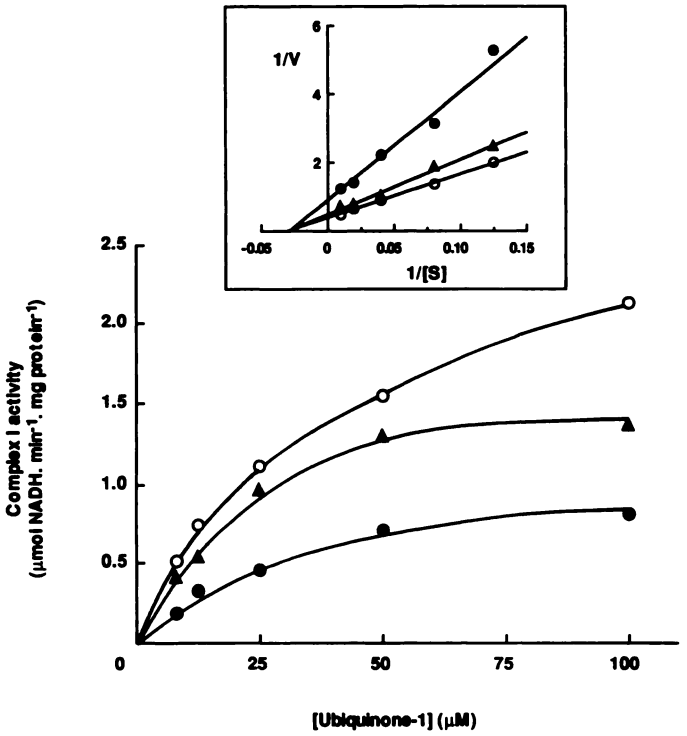

Fig. 4. Kinetic analysis of the inhibition of SMP Complex I activity by flunarizine. All values are expressed as μmol of NADH · min^{-1} · (mg of protein)$^{-1}$ being the means of triplicate assays in the absence (O) and presence of 3 μM (●) or 10 μM (▲) flunarizine. The *inset* shows a double reciprocal plot of the activity versus UQ$_{-1}$ concentration. The K_m and V_{max} values were calculated using a commercial computer program (Ultrafit, BioSoft).

to 5.35 and 3.75 μmol of cytochrome *c* · min^{-1} · (mg of protein)$^{-1}$ with 100 μM flunarizine and 100 μM cinnarizine, respectively. Similarly, when Complex IV was assayed polarographically, both compounds had a stimulatory effect on the activity at concentrations of 10 μM or greater, from control

TABLE 2

Inhibitions of succinate-cytochrome *c* reductase and Complex II activities in SMP by flunarizine and cinnarizine in the absence and presence of 100 μM UQ$_{-1}$

	No UQ$_{-1}$	+100 μM UQ$_{-1}$
Succinate		
Cytochrome *c* reductase		
[μmol of cyto *c* * min^{-1} * (mg of protein)$^{-1}$]		
Control	0.937	1.550
+10 μM Flunarizine	0.081	1.355
+10 μM Cinnarizine	0.069	1.521
Complex II		
[μmol of DCPIP * min^{-1} * (mg of protein)$^{-1}$]		
Control	0.162	0.443
+10 μM Flunarizine	0.124	0.476
+100 μM Flunarizine	0.069	0.418

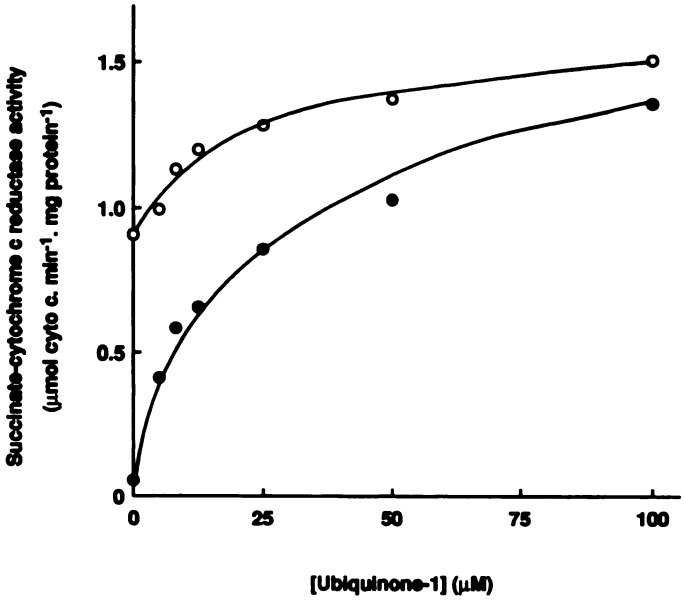

Fig. 5. The protective effect of UQ$_{-1}$ against the inhibition by flunarizine of succinate-cytochrome *c* reductase in rat heart SMP. Means of triplicate assays in the absence (O) and presence (●) of 10 μM flunarizine are shown.

rates of 2.32 μmol of O · min^{-1} · (mg of protein)$^{-1}$, to 3.36 with 10 μM flunarizine, and 3.02 with 100 μM cinnarizine, respectively, with no effects at lower concentrations.

The effects of these inhibitions on superoxide generation were tested with SMP incubated with 100 μM NADH in the presence of 1 mM adrenaline. As shown in Fig. 6, the rate of NADH oxidation was inhibited progressively by 3 to 100 μM flunarizine, and this led to a parallel increase in the rate of adrenochrome formation. The maximal rate, achieved at 100 μM flunarizine when NADH oxidation was inhibited by 95%, was 4.7 nmol of adrenochrome · min^{-1} · (mg of protein)$^{-1}$. This was not significantly different from the rate of 4.4 nmol of adrenochrome · min^{-1} · (mg of protein)$^{-1}$ obtained in the presence of 2.5 μM rotenone when the NADH oxidation rate was decreased by 99%. We also noted that with lower concentrations of flunarizine and, thus, with little inhibition of NADH oxidation, an increase in superoxide generation over base rates still was observed. This increase was present as long as NADH was still being oxidised, i.e., until all the added NADH had been consumed. This suggests that even at low concentrations,

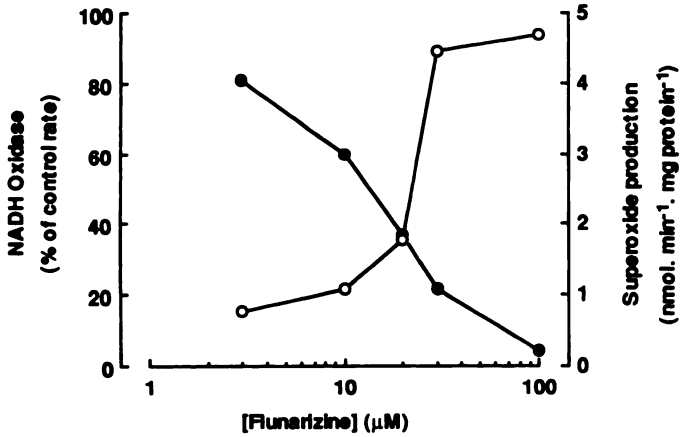

Fig. 6. Inhibition of NADH oxidation and stimulation of superoxide formation by flunarizine. The rates of NADH oxidation (●) and superoxide formation (○) were measured in 100 μg of SMP incubated at 30° in 250 mM sucrose/10 mM Tris (pH 7.4) with 100 μM NADH, 1 mM adrenaline, and 10 μM adrenochrome. The control rate of NADH oxidation was 0.61 μmol of NADH · min^{-1} · (mg of protein)$^{-1}$.

and hence, at only slight inhibition, flunarizine is associated with free radical formation.

Discussion

The experimental induction of a parkinson-like syndrome by the administration of the neurotoxin MPTP is apparently caused by the specific inhibition of mitochondrial Complex I by a metabolite of this compound, which also binds to the dopamine transporter (15). Furthermore, stereotaxic administration into rat brain of specific Complex I inhibitors, such as rotenone and amytal, damaged the nigrostriatal cells in a way that is similar to that caused by MPP^+ (24). Using methods similar to those used to elucidate the mode of action of MPTP, we have investigated the effects of cinnarizine and flunarizine on mitochondrial activities to determine whether the side effects of these compounds may be caused by interference with mitochondrial function.

Both compounds inhibited respiration in intact rat liver mitochondria, with small effects on oxidative phosphorylation, with substrates entering at Complexes I and II of the respiratory chain, but not at Complex IV. In submitochondrial particles, Complexes I and II were inhibited specifically by both cinnarizine and flunarizine. The inhibition of Complex I occurred at a site between the site of NADH-dependent ferricyanide reduction and the ubiquinone-binding site, which is similar to the action of MPP^+ (23). Indeed, this section of Complex I appears to be susceptible to several different species of inhibitor, including rotenone, piericidin A, and amytal (23). This inhibition of Complex I was not competitive with respect to ubiquinone. In contrast, the inhibition of Complex II appears to be caused by competition with ubiquinone, both the endogenous form, which is primarily UQ_{-9} in the rat (25) as evidenced by the inhibition of succinate-cytochrome *c* reduction, and the exogenous UQ_{-1} in the Complex II assay, i.e., succinate-dependent DCPIP reduction.

Therefore, cinnarizine and flunarizine resemble MPP^+ in inhibiting mitochondrial respiratory complexes, particularly Complex I. MPP^+ is presumed to induce parkinsonism by inhibition of mitochondrial respiration in dopaminergic cells of the substantia nigra, hence causing cell death. The specificity for the dopaminergic cells has been proposed to be caused by the selective binding of MPP^+ to the receptors of the dopaminergic uptake system (15). We have no data concerning the actual concentrations of cinnarizine and flunarizine in the brain, or whether these are high enough to explain their parkinson-inducing effects, particularly because the critical factor would be the concentrations within the substantia nigra. Flunarizine interacts with the both the D_2 and, to a lesser extent, the D_1 dopamine receptors (26). Indeed, a specific receptor for piperazine derivatives has been demonstrated in brain membranes (27, 28). It is also interesting to note that the most commonly used marker of the glycoprotein dopamine transporter in the striatum (GBR-12,935) is itself a piperazine-based compound (29). It would be interesting, therefore, to determine whether such interaction could lead to some concentration or uptake of cinnarizine and flunarizine in dopaminergic cells, leading to a subsequent inhibition of mitochondrial respiration with both NAD^+- and FAD^+-dependent substrates. MPP^+ is a potent inhibitor of NADH-dependent oxidations in intact mitochondria, although it is a relatively weak inhibitor of Complex I in disrupted mitochondria or SMP ($K_i \sim 10$ mM) [14]. This is because high levels of MPP^+ within the mitochondrial matrix are required to achieve effective inhibition, the binding site on Complex I being on the inner surface of the mitochondrial inner membrane. Cinnarizine and flunarizine are much more potent inhibitors ($K_i \sim 5$ μM) of Complex I, so concentration within the mitochondrial matrix would be a less important factor for them to display an inhibitory effect. We have not studied mitochondrial uptake of these drugs; indeed, their lipophilic nature suggests that they will remain associated with the membranes. However, respiration in intact liver mitochondria was inhibited, albeit using higher concentrations than those required with submitochondrial particles, showing that the drugs do have access to their binding sites.

More recently it has been suggested that MPP^+ has a biphasic effect on Complex I. The initial inhibition by MPP^+ is readily reversible by simple dilution and washing of the SMP (30). However, longer periods of incubation of SMP with MPP^+ lead to an irreversible inhibition, which has been ascribed to oxidative damage to the complex. Mitochondria are an important source of free radicals, and SMP inhibited by MPP^+ have been shown to generate free radicals when incubated with NADH (31). Indeed, this has been suggested to be the mechanism by which MPP^+ causes permanent inhibition of Complex I *in vivo* (30). We have shown that flunarizine, like rotenone, can increase superoxide production in SMP incubated with NADH. Because such highly reactive radicals presumably would react with susceptible components in their immediate vicinity, this may be another mechanism by which flunarizine decreases the respiratory capacity through oxidative damage to the respiratory chain.

Both cinnarizine and flunarizine are in widespread use, not only in the treatment of severe neurological disorders, but also for relatively minor complaints such as travel sickness. Yet their use has been associated only rarely with the induction of parkinsonism. Although this may be because of differences in the dosages used and the frequency of administration, we should remember also that idiopathic parkinsonism and the cinnarizine- and flunarizine-induced conditions occur prevalently in elderly patients. This could be explained by the age-dependent decreases in mitochondrial respiratory chain activities that

have been demonstrated in various tissues, including liver (32), skeletal muscle (33, 34), and brain (34), which particularly affects Complex I (34). This ageing effect may be associated with the accumulation of mitochondrial DNA deletions (10, 34). It has been suggested that idiopathic parkinsonism may be the result of another insult to Complex I by an endogenous inhibitor (35) or an environmental xenobiotic (36) on top of an age-related loss of Complex I activity, sufficient to decrease the mitochondrial respiration to nonviable levels. Cinnarizine and flunarizine may be representative of such xenobiotics. If the dopaminergic cells of the substantia nigra are selectively affected because of the affinity of piperazines for the dopamine transporter, this would specifically cause the induction of parkinson-like symptoms, rather than a generalized depression of brain respiration. This hypothesis remains to be tested, but should be borne in mind while considering the possible direct effects of the piperazines on dopamine transmission and absorption caused by their affinities for the dopamine transporter.

After this study had been submitted for publication, we received the paper of Burkhardt *et al.* (37), in which they described the inhibition of Complex I in rat brain mitochondria by neuroleptic medications, including haloperidol, chlorpromazine, and thiothixine. These compounds, like flunarizine and cinnarizine, have been associated with extrapyramidal side effects. Furthermore, this group has shown that the Complex I activity in platelets from patients undergoing neuroleptic therapy is depressed significantly. Thus, our studies complement theirs in describing inhibition of mitochondrial respiratory functions by medicaments that provoke parkinson-like effects. This may be further evidence for a mitochondrial role in idiopathic Parkinsonism.

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