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# INHIBITION OF COMPLEX I BY ISOQUINOLINE DERIVATIVES STRUCTURALLY RELATED TO 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)

KEVIN ST. P. McNAUGHT,* ULRIKE THULL,† PIERRE-ALAIN CARRUPT,† COSIMO ALTOMARE,‡ SAVERIO CELLAMARE,‡ ANGELO CAROTTI,‡ BERNARD TESTA,† PETER JENNER*§ and C. DAVID MARSDEN‖

*Neurodegenerative Diseases Research Centre, Pharmacology Group, Biomedical Sciences Division, King's College, London, U.K.; †Institut de Chimie Thérapeutique, Ecole de Pharmacie, BEP, Université de Lausanne, Lausanne, Switzerland; ‡Dipartimento Farmaco-chimico, Università degli Studi di Bari, Bari, Italy; and ‖University Department of Clinical Neurology, Institute of Neurology, National Hospital for Neurology & Neurosurgery, Queen Square, London, U.K.

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**Abstract**—Mitochondrial respiratory failure secondary to complex I inhibition may contribute to the neurodegenerative process underlying nigral cell death in Parkinson's disease (PD). Isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 1-methyl-4-phenylpyridinium ($MPP^+$) may be inhibitors of complex I, and have been implicated in the cause of PD as endogenous neurotoxins. To determine the potency and structural requirements of isoquinoline derivatives to inhibit mitochondrial function, we examined the effects of 22 neutral and quaternary compounds from three classes of isoquinoline derivatives (11 isoquinolines, 2 dihydroisoquinolines, and 9 1,2,3,4-tetrahydroisoquinolines) and $MPP^+$ on the enzymes of the respiratory chain in mitochondrial fragments from rat forebrain. With the exception of norsalsolinol and N,*n*-propylisoquinolinium, all compounds inhibited complex I in a time-independent, but concentration-dependent manner, with $IC_{50}$s ranging from 0.36–22 mM. Several isoquinoline derivatives were more potent inhibitors of complex I than 1-methyl-4-phenylpyridinium ion ($MPP^+$) ($IC_{50}$ = 4.1 mM), the most active being N-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline ($IC_{50}$ = 0.36 mM) and 6-methoxy-1,2,3,4-tetrahydroisoquinoline ($IC_{50}$ = 0.38 mM). 1,2,3,4-Tetrahydroisoquinoline was the least potent complex I inhibitor ($IC_{50} \approx 22$ mM). At 10 mM, only isoquinoline (23.1%), 6,7-dimethoxyisoquinoline (89.6%), and N-methylsalsolinol (34.8%) inhibited ($P < 0.05$) complex II–III, but none of the isoquinoline derivatives inhibited complex IV. There were no clear structure-activity relationships among the three classes of isoquinoline derivatives studied, but lipophilicity appears to be important for complex I inhibition. The effects of isoquinoline derivatives on mitochondrial function are similar to those of MPTP/$MPP^+$, so respiratory inhibition may underlie their reported neurotoxicity.

*Key words:* Complex I, Parkinson's disease; 1,2,3,4-tetrahydroisoquinoline; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 1-methyl-4-phenylpyridinium; mitochondria

The cause of nigral cell death in PD remains unknown, but studies of brain tissue from patients dying with PD suggest that oxidative stress and impaired mitochondrial function may contribute to the neurodegenerative process occurring in the substantia nigra [20]. Thus, there is evidence for increased lipid peroxidation [9], increased iron levels but decreased ferritin content [8], increased mitochondrial superoxide dismutase activity [44], decreased levels of reduced glutathione [49], inhibition of NADH ubiquinone reductase (complex I) activity [47], and reduced immunostaining for α-ketoglutarate dehydrogenase [28] in substantia nigra in PD.

The biochemical basis of the complex I deficiency in PD is unknown and there is no compelling evidence for a defect in any of its subunits, encoding mechanism, or mitochondrial DNA [46]. However, complex I has more than 40 subunits, and it remains unknown whether there is a defect in its nuclear genes. Alternatively, the complex I defect could result from the actions of an environmental or endogenous toxic substance. Indeed, MPTP is toxic to dopaminergic neurones via its active metabolite $MPP^+$, which selectively inhibits complex I of the electron transport chain following its energy-dependent accumulation within mitochondria [39, 40]. MPTP, $MPP^+$, or close pyridine analogs are, however, unlikely to be responsible for PD since, for example, antibodies to these compounds show no immunoreactivity in substantia nigra in PD [18, 19]. Rather, isoquinoline derivatives (e.g. 1,2,3,4-tetrahydroisoquinolines) structurally related to MPTP or $MPP^+$ are formed within the brain through condensation of biogenic amines with aldehydes, and have emerged as candidate endogenous neurotoxins [5].

Isoquinoline derivatives were first characterised as inhibitors of tyrosine hydroxylase [31], and have now been reported to possess many of the cytotoxic characteristics of MPTP. Some isoquinoline derivatives, N-methyl-1,2,3,4-tetrahydroisoquinoline (N-Me-THIQ) for example, are metabolised by MAO-B to produce the corresponding N-methylisoquinolinium cations [32] that have been shown to be substrates for the dopamine re-uptake system [16]. Cytotoxicity of the isoquinoline derivatives, N-methylisoquinolinium ($N\text{-Me-IQ}^+$), THIQ, and

§ Corresponding author Professor Peter Jenner, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Road, London, SW3 6LX, U.K. Tel. 071-333-4716; FAX 071-376-4736.

¶ Abbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; $MPP^+$, 1-methyl-4-phenylpyridinium ion; PD, Parkinson's disease; THIQ, 1,2,3,4-tetrahydroisoquinoline; MAO-B, monoamine oxidase B.

N-Me-THIQ has been demonstrated in cultured mesencephalic neurones [34, 35] and on direct intranigral application *in vivo* [45]. 2-Methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline depletes catecholamines in rat brain [25], and systemic administration of the putative neurotoxin, THIQ, to non-human primates produces parkinsonism-like motor disorders with neurochemical, histological, and behavioural changes similar to those observed with MPTP [54, 30].

The mechanism underlying the neurotoxicity of isoquinoline derivatives is unclear, but may be similar to that of $MPP^+$. Indeed, previous studies suggest that these compounds are inhibitors of complex I [50–52]. Suzuki and colleagues [50] reported that THIQ concentration-dependently inhibited the activity of complex I with an $IC_{50}$ (2.25 mM) similar to that of $MPP^+$ (3.2 mM), but had no effect on complexes II, III, or IV in mouse brain mitochondrial fragments. Its oxidised congener N-Me-$IQ^+$ also selectively inhibited complex I, but more potently ($IC_{50}$ = 500 μM) than the parent compound or $MPP^+$ [52]. In contrast, the methylated derivative of THIQ, N-Me-THIQ, was less potent ($IC_{50}$ = 6.5 mM) than both compounds in inhibiting complex I activity.

Although complex I inhibition may underlie the reported neurotoxicity of isoquinoline derivatives, their relevance as potential mitochondrial toxins remain unclear. In particular, there has been no study of a series of structurally closely related isoquinoline derivatives that could be used to correlate the effects of lipophilicity and structural elements on mitochondrial inhibition. We now examine the potency and structural requirements of isoquinoline derivatives to inhibit mitochondrial function by studying the effects of 22 neutral and quaternary compounds from three classes of isoquinoline derivatives (11 isoquinolines, 2 dihydroisoquinolines, and 9 1,2,3,4-tetrahydroisoquinolines) on the enzymes of the respiratory chain in mitochondrial fragments from rat forebrain.

## MATERIALS AND METHODS

### *Materials*

$MPP^+$ iodide (**w**) was obtained from Research Biochemicals Inc. (U.S.A.). Cytochrome c was obtained from Boehringer (Germany). Ficoll-400 and essentially fatty acid free bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (Poole, U.K.). A 7.5% (w/w) and 12% (w/w) solution of Ficoll-400 was prepared as previously described [24]. Ubiquinone-1 was a gift from Eisai Pharmaceutical Company (Tokyo, Japan), and was prepared as detailed elsewhere [3]. The reduced form of cytochrome c was prepared as previously described [6]. All other chemicals were of analytical grade obtained from commercial sources.

The substituted isoquinolines (**d, f, h, j**) were prepared as previously described [10, 15]. N-Methylisoquinolinium iodides (**b, e, g, i, k**) were prepared from the corresponding isoquinolines and methyl iodide (*n*-propyl iodide for compound **c**) by standard procedures. 1,2,3,4-Tetrahydroisoquinolines (**p, r, s**) were prepared by catalytic reduction ($H_2$/Pt) of the corresponding isoquinolines in acetic acid (50–80°C, 24–48 hr). N-Methyl-6-methoxy-1,2-dihydroisoquinoline (**l**) was prepared by reduction of compound **e** with lithium aluminium hydride in anhydrous tetrahydrofuran at room temperature (6 hr) and isolated as hydrochloride. Catalytic reduction (Pt) of compound **l** in anhydrous ethanol (at room temperature, 6 hr) gave N-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**r**) in 80% yield. 6,7-Dimethoxy-1-styryl-3,4-dihydroisoquinoline hydrochloride (**m**) was obtained as a gift from Prof. H. D. Höltje (Freie Universität, Berlin, Germany). The salsolinols (**t, u, v**) were a gift from Dr. B. Goodwin (King's College London), and were synthesised by standard procedures using dopamine and acetaldehyde (aq., pH 7.0). The other isoquinoline derivatives (**a, n, o, q**) and chemicals used in the synthetic procedures were obtained from Aldrich Chemical Co. (Milwaukee, WI). The chemical structure and purity of the isoquinoline derivatives (Fig. 1) were analysed by NMR, IR, elemental analysis, HPLC, and GC-MS.

### *Isolation of rat brain mitochondria*

For each experiment, non-synaptic (free) mitochondria were isolated from the forebrains of four male Wistar rats (200–250 g; Bantin & Kingman, Hull, U.K.) using the method of Lai and Clark [24]. Rats were killed by stunning and cervical dislocation, the brains immediately removed and the forebrains rapidly dissected on ice. All subsequent procedures were performed at 4°C in isolation medium containing 320 mM sucrose, 1 mM $K^+$EDTA, 10 mM Tris-HCl, pH 7.4, as previously described [24]. Mitochondrial isolates were suspended in isolation medium to a final protein concentration of 10 mg/mL determined by the folin phenol method of Lowry [26] using BSA as protein standard, then subjected to three cycles of freeze/thawing immediately before enzyme assays to produce mitochondrial fragments, thus ensuring maximal enzyme activities and inhibition. There was no significant change in either the protein content or enzyme activities of the mitochondrial fragments when kept on ice for the duration of the experiments or when stored at −70°C for several weeks (data not shown).

### *Mitochondrial enzyme assays*

Enzyme assays were conducted at 37°C in a final reaction volume of 1.0 mL and monitored spectrophotometrically using a Shimadzu UV-2101 recording spectrophotometer equipped with an isothermal multicell holder (Shimadzu, Japan). NADH ubiquinone-1 reductase (complex I; EC 1.6.99.3), succinate cytochrome c reductase (complex II–III; EC 1.8.3.1) and cytochrome c oxidase (complex IV; EC 1.9.3.1) were assayed as previously described [23]. Control experiments were conducted in which rotenone (10 μM) or antimycin A (10 μg) was added to the complex I and complex II reaction mixtures, respectively, 5 min after the reaction was initiated and followed for a further 5 min. The activity of complex I (rotenone-sensitive rate) and complex II-III (antimycin A-sensitive rate) were calculated as the total rate (rate before inhibitor added) minus the inhibitor-insensitive rate (rate after inhibitor added). Rotenone-sensitive and antimycin A-sensitive values were approximately 90% and 70% of total rates. All measurements were made against a reference (blank) cuvette containing the exact content of the experimental cuvette, except that distilled water was added instead of the reaction initiator.

### *Assessment of mitochondrial enzyme inhibition*

In experiments to determine the effects of isoquinoline derivatives or $MPP^+$ at 10 mM on the activities of

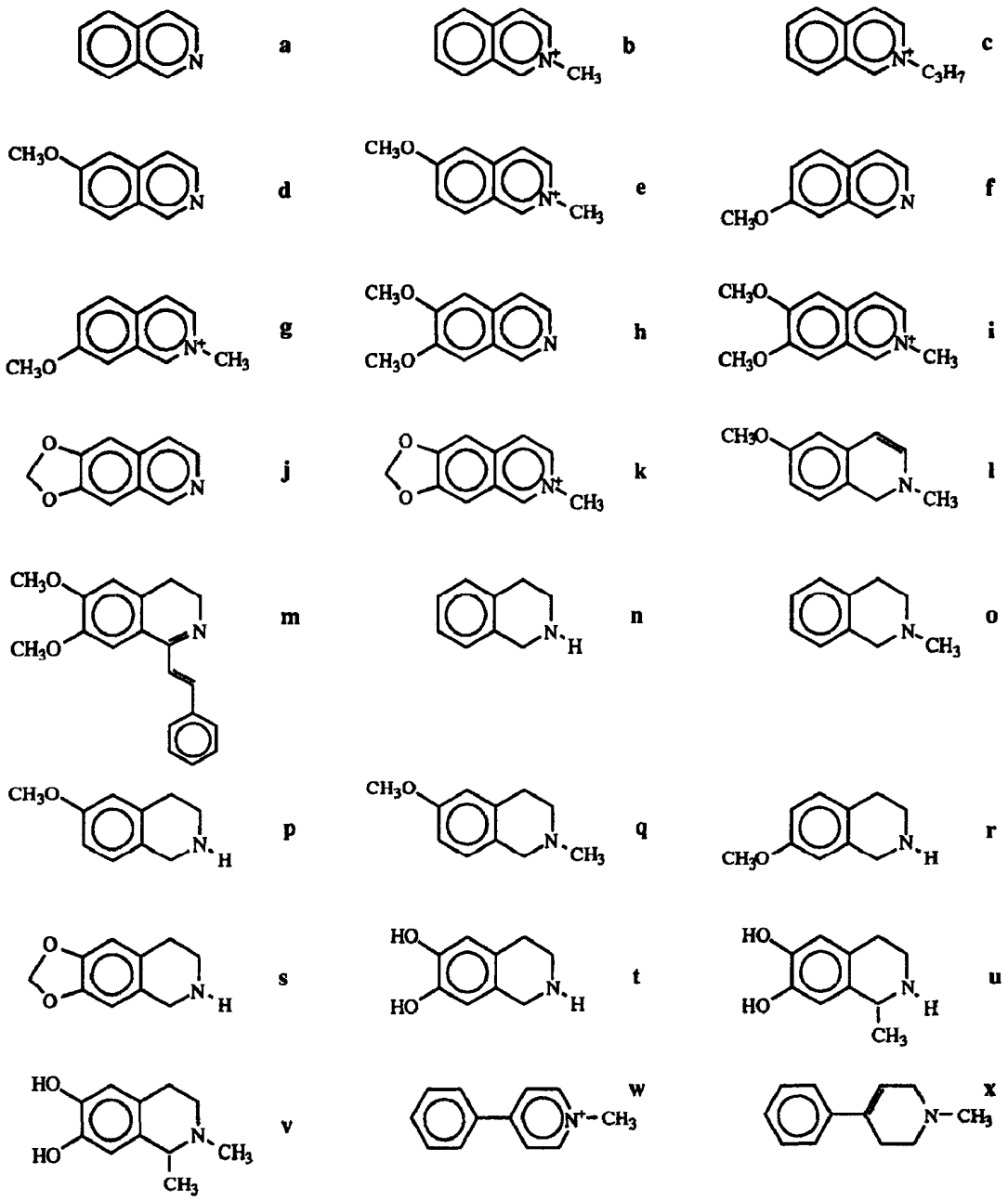


Fig. 1. Structures of the isoquinoline derivatives. (a) Isoquinoline (IQ); (b) N-Methylisoquinolinium iodide (N-Me-IQ$^+$); (c) N-*n*-Propylisoquinolinium iodide (N-Pr-IQ$^+$); (d) 6-Methoxyisoquinoline (6-OMe-IQ); (e) N-Methyl-6-methoxyisoquinolinium iodide (N-Me-6-OMe-IQ$^+$); (f) 7-Methoxyisoquinoline (7-OMe-IQ); (g) N-Methyl-7-methoxyisoquinolinium iodide (N-Me-7-OMe-IQ$^+$); (h) 6,7-Dimethoxyisoquinoline (6,7-diOMe-IQ); (i) N-Methyl-6,7-dimethoxyisoquinolinium iodide (N-Me-6,7-diOMe-IQ$^+$); (j) 6,7-Methylenedioxyisoquinoline hydrochloride (6,7-$OCH_2O$-IQ); (k) N-Methyl-6,7-methylenedioxyisoquinolinium iodide (N-Me-6,7-$OCH_2O$-IQ$^+$); (l) N-Methyl-6-methoxy-1,2-dihydroisoquinoline hydrochloride (N-Me-6-OMe-1,2-DHIQ); (m) 6,7-Dimethoxy-1-styryl-3,4-dihydroisoquinoline hydrochloride (6,7-diOMe-1-S-3,4-DHIQ); (n) 1,2,3,4-Tetrahydroisoquinoline hydrochloride (THIQ); (o) N-Methyl-1,2,3,4-tetrahydroisoquinoline (N-Me-THIQ); (p) 6-Methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (6-OMe-THIQ); (q) N-Methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (N-Me-6-OMe-THIQ); (r) 7-Methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (7-OMe-THIQ); (s) 6,7-Methylenedioxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (6,7-$OCH_2O$-THIQ); (t) 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (norsalsolinol); (u) 1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (salsolinol); (v) 1,2-Dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (N-Me-salsolinol); (w) 1-Methyl-4-phenylpyridinium iodide (MPP$^+$); and (x) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

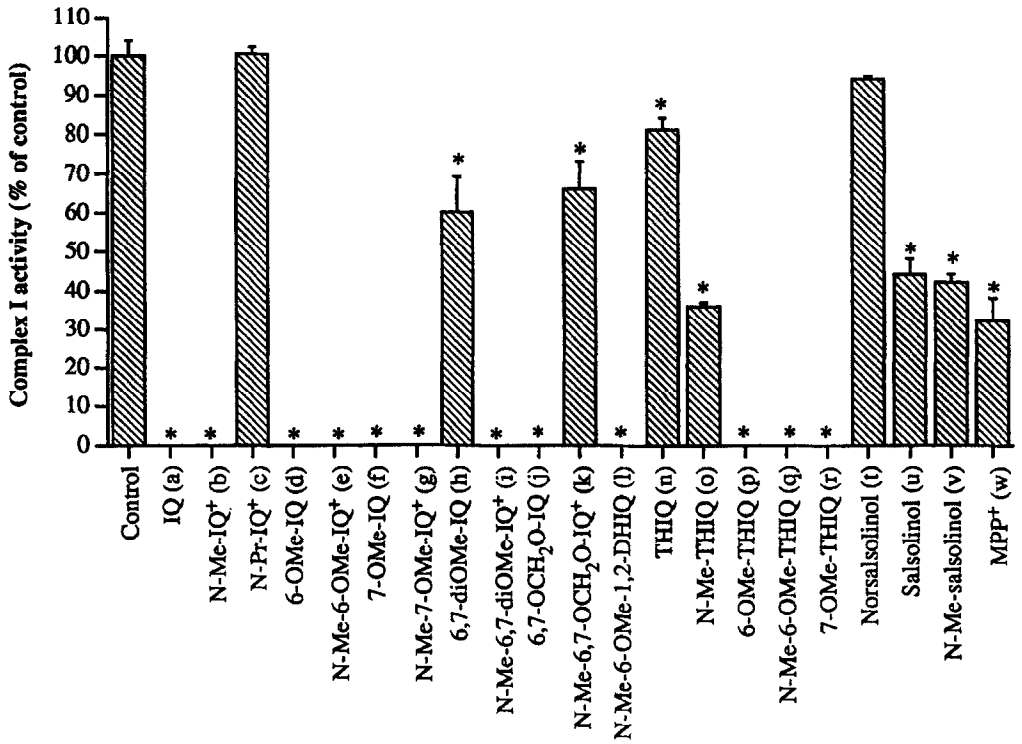


Fig. 2. Effects of isoquinoline derivatives and $MPP^+$ (10 mM) on complex I activity. The isoquinoline derivatives or $MPP^+$ (10 mM) were added to the complex I reaction mixture with the enzyme substrates and allowed to equilibrate for 1 min before the reaction was initiated by the addition of mitochondrial fragments. 6,7-diOMe-1-S-3,4-DHIQ (m) is approximately 87% protonated. Data are presented as mean ± SEM ($n$ = 3). *$P < 0.05$ (Mann-Whitney $U$-test).

complexes I, II–III, and IV, each compound was added to the respective reaction mixture with the enzyme substrates and allowed to equilibrate for 1 min before the reaction was initiated by the addition of mitochondrial fragments (1–50 μg). To determine the effects of prolonged incubation of complex I with the isoquinoline derivatives or $MPP^+$ on inhibitory potency, each compound (10 mM) was added to the complex I reaction mixture with mitochondrial fragments (1–50 μg) and allowed to incubate for up to 30 min before the reaction was initiated with ubiquinone-1. In experiments to determine the concentration-dependent inhibition of complex I by the isoquinoline derivatives or $MPP^+$, the compounds were added to the complex I reaction mixture at concentrations ranging from 0.1–20 mM.

In all enzyme assays, except for complex IV, reaction rates were measured for 10 min and were linear for at least 5 min, then progressively declined. All enzymatic rates were determined from the initial portion of the activity profile and expressed as amount (nmol) of substrate utilised per minute (min) per milligram (mg) of mitochondrial protein (nmol/min/mg). For complex IV, since the reaction is first order with respect to cytochrome c, the pseudo first-order rate constant ($k$) was determined following linear transformation of the reaction curves ($k$/min/mg). All assays were performed in triplicate, and data presented as mean ± SEM.

Ethanol was used as a solvent for several of the isoquinoline derivatives, and some compounds were used as the iodides or chlorides. Consequently, as a control, the effects of 10 μL ethanol, 10 mM NaI, or 10 mM

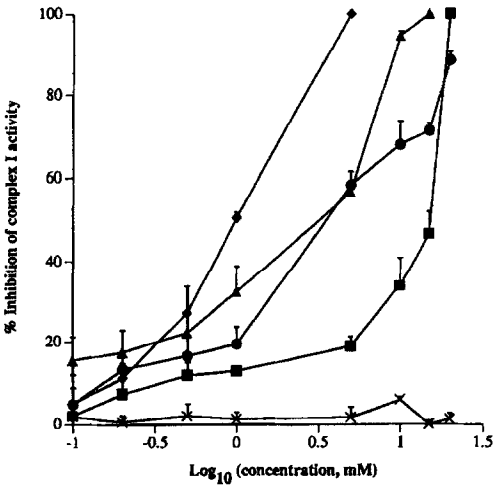


Fig. 3. Concentration-dependent inhibition of complex I activity by isoquinoline derivatives and $MPP^+$. The isoquinoline derivatives or $MPP^+$ (0.1–20 mM) were added to the complex I reaction mixture with the enzyme substrates and allowed to equilibrate for 1 min before the reaction was initiated by the addition of mitochondrial fragments. Plots of percentage (%) inhibition of complex I activity vs $Log_{10}$ (concentration of compound, mM) were constructed. Data are presented as mean ± SEM ($n$ = 3).
Symbols: ♦, 7-OMe-IQ (f); ■, N-Me-6,7-$OCH_2O$-$IQ^+$ (k); ▲, N-Me-THIQ (o); ×, Norsalsolinol (t); ●, $MPP^+$ (w).

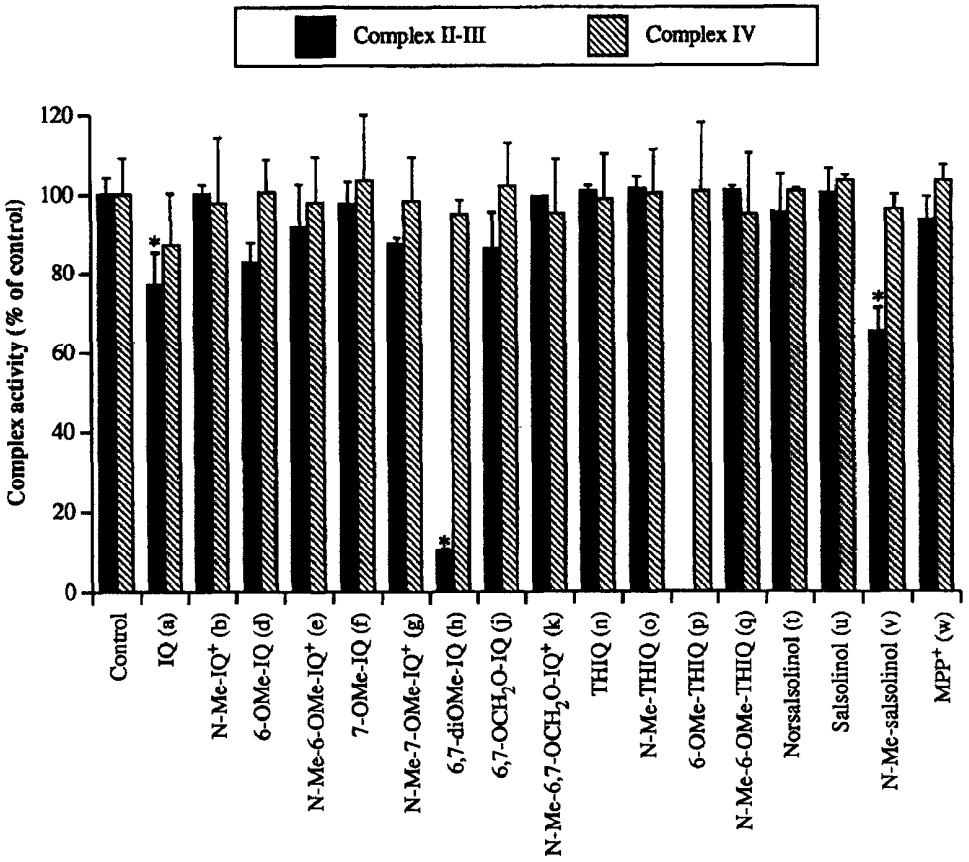


Fig. 4. Effects of isoquinoline derivatives and $MPP^+$ (10 mM) on complex II–III and complex IV activity. The isoquinoline derivatives or $MPP^+$ (10 mM) were added to the respective reaction mixture with the enzyme substrates and allowed to equilibrate for 1 min before the reaction was initiated by the addition of mitochondrial fragments. The isoquinoline derivatives **c, i, l, m, r,** and **s** were not studied. Data are presented as mean + SEM ($n = 3$).

*$P < 0.05$ (Mann-Whitney $U$-test).

NaCl on the activities of the mitochondrial enzymes were examined. These compounds had no effect on the activity of any of the mitochondrial enzymes (data not shown).

*Lipophilicity measurements*

Distribution coefficients in a 1-octanol/water system were measured by Centrifugal Partition Chromatography (CPC), mutually saturated 1-octanol and 0.01 M phosphate buffer (pH 7.4) being the stationary and mobile phases, respectively. Measurements were performed (25 ± 1°C) using apparatus and procedures described elsewhere [1, 2, 11]. For ionisable solutes, correction for ionisation afforded partition coefficients. Calculated log P values were obtained using the *CLOGP* 3.54 software [14]; this system is based on hydrophobic fragmental constants and correction factors elaborated by Hansch and Leo [13].

*Statistical analysis*

Results were analysed statistically using the Mann-Whitney $U$-test. Linear regression analysis was used to determine the correlation of log P and $pIC_{50}$ for complex I inhibition.

## RESULTS

*Complex I*

At a concentration of 10 mM, norsalsolinol (**t**) and N-Pr-$IQ^+$ (**c**) had no effect ($P > 0.05$) on complex I activity, but complete inhibition was produced by IQ (**a**), N-Me-$IQ^+$ (**b**), 6-OMe-IQ (**d**), N-Me-6-OMe-$IQ^+$ (**e**), 7-OMe-IQ (**f**), N-Me-7-OMe-$IQ^+$ (**g**), N-Me-6,7-di-OMe-$IQ^+$ (**i**), 6,7-$OCH_2$O-IQ (**j**), N-Me-6-OMe-1,2-DHIQ (**l**), 6-OMe-THIQ (**p**), N-Me-6-OMe-THIQ (**q**) and 7-OMe-THIQ (**r**) (Fig. 2). The other isoquinoline derivatives partially inhibited complex I activity by 19–68% ($P < 0.05$) and $MPP^+$ (**w**) inhibited complex I activity by 69% ($P < 0.05$) (Fig. 2). The active isoquinoline derivatives inhibited complex I activity in a time-independent (data not shown) but concentration-dependent manner, with $IC_{50}$s ranging from 0.36–22 mM (Fig. 3; Table 2). N-Me-6-OMe-THIQ (**q**) ($IC_{50} = 0.36$ mM) and 6-OMe-THIQ (**p**) ($IC_{50} = 0.38$ mM) were the most potent inhibitors of complex I activity, while THIQ (**n**), the least potent compound, failed to produce 50% inhibition. N-Me-THIQ (**o**) and $MPP^+$ (**w**) had similar potencies with $IC_{50}$s of 4.3 mM and 4.1 mM, respectively.

*Complex II–III*

At 10 mM, only IQ (**a**), 6,7-diOMe-IQ (**h**), and N-Me-salsolinol (**v**) inhibited complex II–III activity, produc-

Table 1. Octanol/water partition coefficients of the investigated isoquinoline derivatives and $MPP^+$

| Compounds | Log P |
|---|---|
| Isoquinolinium ions | |
| N-Me-$IQ^+$ (**b**) | −2.75* |
| N-Pr-$IQ^+$ (**c**) | −2.30 |
| N-Me-6-OMe-$IQ^+$ (**e**) | −1.83 |
| N-Me-7-OMe-$IQ^+$ (**g**) | −1.98 |
| N-Me-6,7-diOMe-$IQ^+$ (**i**) | −2.06 |
| N-Me-6,7-$OCH_2O$-$IQ^+$ (**k**) | −2.24 |
| Isoquinolines | |
| IQ (**a**) | 2.08 |
| 6-OMe-IQ (**d**) | 1.98 |
| 7-OMe-IQ (**f**) | 2.01 |
| 6,7-diOMe-IQ (**h**) | 1.68 |
| 6,7-$OCH_2O$-IQ (**j**) | 1.75 |
| 1,2,3,4-tetrahydroisoquinolines | |
| THIQ (**n**) | 1.71 |
| N-Me-THIQ (**o**) | 2.14 |
| 6-OMe-THIQ (**p**) | 1.59‡ |
| N-Me-6-OMe-THIQ (**q**) | 2.32 |
| 7-OMe-THIQ (**r**) | 1.59‡ |
| 6,7-$OCH_2O$-THIQ (**s**) | 1.03‡ |
| Norsalsolinol (**t**) | 0.34‡ |
| Salsolinol (**u**) | 0.86‡ |
| N-Me-salsolinol (**v**) | 1.67‡ |
| Dihydroisoquinolines | |
| 6,7-diOMe-1-S-3,4-DHIQ (**m**) | 3.39§ |
| N-Me-6-OME-1,2-DHIQ (**l**) | |
| Others | |
| $MPP^+$ (**w**) | −2.28* |
| MPTP (**x**) | 2.71† |

* Taken from Altomare *et al.* (1991); cited with permission.
† Taken from Altomare *et al.* (1992); cited with permission.
‡ Calculated by CLOGP 3.54 software.
§ Log $D_{(pH\ 7.4)}$ = 2.52; UV-Spectrophotometrically determined $pK_a$ = 8.23 ± 0.09.

ing a 89.6, 34.8, and 23.1% inhibition, respectively (Fig. 4). The other isoquinoline derivatives and $MPP^+$ (**w**) did not inhibit complex II–III activity (Fig. 4).

*Complex IV*

None of the compounds examined had any effect on complex IV activity at 10 mM (Fig. 4).

*Structure-Activity relationships*

The octanol-water partition coefficients (log P) of the isoquinoline derivatives and $MPP^+$ (**w**) are shown in Table 1. There was no evident lipophilicity-activity relationship for the complete series of compounds examined for inhibition of complex I activity. There was, however, a direct relationship between log P and $pIC_{50}$ ($r^2$ = 0.92) within the subset of methoxylated N-methylisoquinolinium ions (i.e. N-Me-6-OMe-$IQ^+$ (**e**), N-Me-7-OMe-$IQ^+$ (**g**), 6,7-diOMe-$IQ^+$ (**h**), and N-Me-6,7-$OCH_2O$-$IQ^+$ (**k**)) (Fig. 5).

## DISCUSSION

The process underlying degeneration of the dopaminergic neurones of the nigrostriatal pathway in PD is unknown. The discovery of a specific complex I defect in the substantia nigra in PD and the ability of $MPP^+$ to inhibit complex I activity suggest that failure of mitochondrial energy metabolism secondary to complex I inhibition may be responsible for cell death in PD. One possibility is that mitochondrial dysfunction occurs as a result of the actions of an environmental or endogenous toxic substance. Isoquinoline derivatives structurally related to MPTP have emerged as candidate endogenous neurotoxins with some evidence for inhibition of complex I activity as their mechanism of action. However, the mitochondrial toxicity of isoquinoline derivatives remains unclear since there has been no comprehensive study of these compounds to determine selectivity, potency, and structural requirements for enzyme inhibition. Therefore, in this study we have examined the effects of 22 structurally closely related isoquinoline derivatives on the enzymes of the respiratory chain in rat forebrain mitochondrial fragments.

Most of the compounds examined displayed concentration-dependent inhibition of complex I activity, and some were markedly more potent than $MPP^+$ (**w**). Some of these compounds have previously been investigated by others, and overall the present results are in good agreement with these reports. For example, N-Me-THIQ (**o**) and $MPP^+$ (**w**) were shown to be equipotent inhibitors of complex I with $IC_{50}$s of 4.3 mM and 4.1 mM, respectively. These values are close to the $IC_{50}$s of 6.5 mM [52] and 3.2 mM [29] reported earlier. Similarly, the $IC_{50}$ of IQ (**a**) and N-Me-$IQ^+$ (**b**) in this study was approximately 0.8 mM and 1.3 mM, in agreement with 1 mM [45] and approx. 0.7 mM [50], respectively, reported previously. However, we found that THIQ (**n**) ($IC_{50}$ = 22 mM) was not as potent as reported by Suzuki and colleagues [52] ($IC_{50}$ = 2.25 mM). The reason for this discrepancy is unclear, but may arise from the different methodologies used. In our study, mitochondrial isolates were subjected to three cycles of freeze/thawing to produce mitochondrial fragments. Only one cycle was employed in Suzuki's study, so less mitochondrial fragmentation would occur, resulting in some mitochondrial concentration of these compounds.

The inhibitory effects of isoquinoline derivatives and $MPP^+$ (**w**) were highly selective for complex I, since only the neutral compounds, IQ (**a**), 6,7-diOMe-IQ (**h**), and N-Me-salsolinol (**v**) partially inhibited complex II-III (a combined measure of complexes II and III) and none of the compounds inhibited complex IV. These findings are in agreement with reports of the inability of MPTP, $MPP^+$, and related compounds to inhibit these enzymes [29, 50, 52].

There were no clear structure-activity relationships among the three classes of compounds and their effects on complex I activity, but some trends are evident. For complex I inhibition, the degree of aromatisation on the activity of the compound was interesting although inconclusive. Comparing the potencies of N-Me-6-OMe-1,2-DHIQ (**l**) ($IC_{50}$ = 0.54 mM) with N-Me-6-OMe-THIQ (**q**) ($IC_{50}$ = 0.36 mM) and 6-OMe-IQ (**d**) ($IC_{50}$ = 0.5 mM) with 6-OMe-THIQ (**p**) ($IC_{50}$ = 0.38 mM) suggests that the degree of aromatisation influences inhibitory activity, but only two dihydroisoquinolines were investigated. The presence of a quaternary nitrogen was not necessary for complex I inhibition. Thus, the inhibitory activity of the fully aromatic isoquinoline derivatives was not different from that of the corresponding N-methylisoquinolinium cations. Indeed, Sayer and colleagues [45] also found that IQ (**a**) ($IC_{50}$ = 1 mM) was more potent than its quaternary congener, N-Me-$IQ^+$ (**b**) ($IC_{50}$ > 20 mM) and, indeed, $MPP^+$ (**w**)

Table 2. Inhibition of complex I activity by isoquinoline derivatives and MPP†

| Compound | $IC_{50}$ (mM) | Compound | $IC_{50}$ (mM) |
|---|---|---|---|
| IQ (**a**) | 0.75 | 6,7-diOMe-1-S-3,4-DHIQ (**m**) | ND |
| N-Me-IQ† (**b**) | 1.3 | THIQ (**n**) | 22* |
| N-Pr-IQ† (**c**) | ND | N-Me-THIQ (**o**) | 4.3 |
| 6-OMe-IQ (**d**) | 0.5 | 6-OMe-THIQ (**p**) | 0.38 |
| N-Me-6-OMe-IQ† (**e**) | 0.7 | N-Me-6-OMe-THIQ (**q**) | 0.36 |
| 7-OMe-IQ (**f**) | 1.1 | 7-OMe-THIQ (**r**) | 1.1 |
| N-Me-7-OMe-IQ† (**g**) | 0.85 | 6,7-$OCH_2O$-THIQ (**s**) | ND |
| 6,7-diOMe-IQ (**h**) | 15 | Norsalsolinol (**t**) | NA |
| N-Me-6,7-diOMe-IQ† (**i**) | 2.3 | Salsolinol (**u**) | 8.9 |
| 6,7-$OCH_2O$-IQ (**j**) | 0.39 | N-Me-salsolinol (**v**) | 4.6 |
| N-Me-6,7-$OCH_2O$-IQ† (**k**) | 15.4 | MPP† (**w**) | 4.1 |
| N-Me-6-OMe-1,2-DHIQ (**l**) | 0.54 | | |

The isoquinoline derivatives or MPP† (0.1–20 mM) were added to the complex I reaction mixture with the enzyme substrates and allowed to equilibrate for 1 min before the reaction was initiated by the addition of mitochondrial fragments. Plots of percentage (%) inhibition vs concentration of compound were constructed from which the mean $IC_{50}$ (mM) values were determined.

NA: Not attained (failure to reach 50% inhibition).

ND: Not determined.

* Curve extrapolated to obtain approximate $IC_{50}$ value.

($IC_{50}$ = 9.5 mM). The potency of neutral compounds is consistent with the inhibition of complex I shown by pyridine analogs [42, 43, 53, 17, 55, 12]. Furthermore, the rotenoids (e.g. rotenone) and piericidins (e.g. piericidin A), which are potent inhibitors of complex I activity, are also neutral compounds with no obvious structural similarity to pyridines or isoquinoline derivatives. The seven mitochondrially encoded subunits of complex I form part of the hydrophobic shell around the more hydrophilic iron-sulphur proteins constituting the catalytic subunits. Therefore, lipophilic compounds, as opposed to hydrophilic charged species, would more readily gain access to the active site of complex I. Indeed, N-methylation, which acts to increase lipophilicity, increased the inhibitory activity of 1,2,3,4-tetrahydroisoquinolines, as is evident from comparing the potencies of THIQ (**n**) with N-Me-THIQ (**o**) and salsolinol (**u**) with N-Me-salsolinol (**v**). Moreover, Youngster and colleagues [55] and Gluck and colleagues [12] reported that alkylation of $MPP^+$ at the 4'position of the phenyl ring, considerably increases inhibitory potency at complex I in mitochondrial fragments as the length of the alkyl chain increases. Comparing the complex I inhibitory activity of ring-substituted vs unsubstituted isoquinoline congeners shows that methoxylation in position 6 rather than in position 7 increases the activity of the compound. However, double methoxylation (i.e. 6,7-dimethoxy derivatives) is less favourable for activity, whereas the 6,7-dioxymethylene group decreases the activity of the N-methylisoquinolinium cation, N-Me-6,7-$OCH_2O$-$IQ^+$ (**k**), but not that of IQ (**a**). Furthermore, methoxy-substituted tetrahydroisoquinolines, 6-OMe-THIQ (**p**), N-Me-6-OMe-THIQ (**q**), and 7-OMe-THIQ (**r**) are more potent inhibitors than the more hydrophilic catechol isoquinolines (i.e. norsalsolinol (**t**), salsolinol (**u**), and N-Me-salsolinol (**v**)). This again suggests that hydrophobic interactions and/or unfavourable hydrogen-bond donor properties of the substituents in positions 6 and 7 may play a role in the molecular recognition process underlying complex I inhibition. Additional studies on further congeners with a better spread of hydrophobic character are necessary to define the role of lipophilicity in modulating the inhibition of complex I by isoquinoline derivatives. The inability of N-Pr-$IQ^+$ (**c**) to inhibit complex I appears to be a consequence of increased bulkiness of the N-alkyl group (*n*-propyl instead of methyl).

The octanol/water partition coefficients of the isoquinoline derivatives showed that within the THIQ and IQ subsets, the variations of log P essentially reflect the additive contributions of the substituents on the isoquinoline nucleus. In contrast, delocalization of the positive charge due to the electron-donating character of methoxy and methylenedioxy groups is taken into account to rationalize the variation of log P of the isoquinolinium cations. The introduction of $-OCH_3$ or $-OCH_2O-$ groups on N-Me-$IQ^+$ (**b**) resulted in a significant increase in log P values, the increment ranging from 0.51 for N-Me-6,7-$OCH_2O$-$IQ^+$ (**k**) to 0.92 for N-Me-6OMe-$IQ^+$ (**e**) log units. Moreover, a slight but real difference of log P (0.15 log units), which apparently depends on charge delocalization, was observed between the 6-$OCH_3$ (N-Me-6-OMe-$IQ^+$ (**e**)) and the

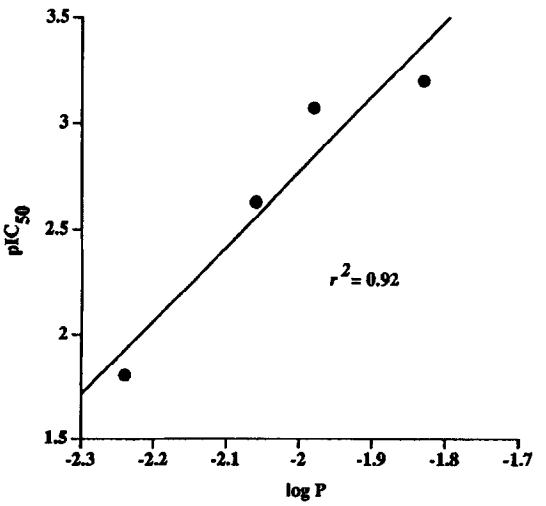


Fig. 5. Plot of log P versus $pIC_{50}$ of substituted N-methylisoquinolinium cations.

7-$OCH_3$ congener (N-Me-7-OMe-IQ$^+$ (**g**)). Indeed, the positive charge must be more delocalized in N-Me-6-OMe-IQ$^+$ (**e**) (log P = −1.83) than in the 7-$OCH_3$ isomer (log P = −1.98) due to the contribution of a para-quinoid mesomeric structure in the former isomer. Comparing log P with $pIC_{50}$ values, no evident lipophilicity-activity relationship emerges considering the whole series of compounds investigated, whereas a direct relation between log P and $pIC_{50}$ within the subset of methoxylated N-methylisoquinolinium cations (Fig. 5) indicates that a decrease in hydrophilicity of these charged compounds could be favourable to complex I inhibition.

Unlike MPTP (**x**), many isoquinoline derivatives, such as THIQ (**n**), N-Me-THIQ (**o**), and salsolinol (**u**) investigated in this study, occur naturally as endogenous alkaloids in the CNS of animals [22] and humans [37]. These compounds are synthesised by Pictet-Spengler condensation of biogenic amines with aldehydes [7], and are metabolised *in vivo*. For example, the putative neurotoxin THIQ (**n**) is methylated by N-methyltransferase to produce N-Me-THIQ (**o**), which is subsequently converted by MAO-B to N-Me-IQ$^+$ (**b**), the isoquinoline derivative most similar to MPP$^+$ (**w**) [32, 33]. Isoquinoline derivatives are widely distributed in the environment, being present in many plants [48], foodstuffs (e.g. cocoa, bananas, etc.), and some alcoholic beverages [27, 36], and may therefore represent an additional means of CNS accumulation, as these compounds are able to cross the blood-brain barrier [21]. Interestingly, the levels of THIQ (**n**) and tetrahydropapaveroline are elevated in the brain [38] and urine [4], respectively, of parkinsonian patients.

In summary, most of the isoquinoline derivatives examined in this study inhibited complex I activity, with some being markedly more potent than MPP$^+$. There were no clear structure-activity relationships among the three classes of isoquinoline derivatives studies, but lipophilicity may be important for complex I inhibition. These findings suggests that the biochemical properties of isoquinoline derivatives are similar to those of MPTP, MPP$^+$, and their analogs in mitochondrial fragments. However, the mitochondrial toxicity of MPP$^+$ depends on active concentration aided by the transmembrane electrochemical gradient [39], so we are now studying the effects of isoquinoline derivatives on respiration in intact mitochondria.

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