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# Effects of Isoquinoline Derivatives Structurally Related to 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) on Mitochondrial Respiration

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**ABSTRACT.** Isoquinoline derivatives exert 1-methyl-4-phenylpyridinium ($MPP^+$)-like activity as inhibitors of complex I and α-ketoglutarate dehydrogenase activity in rat brain mitochondrial fragments. We now examine the ability of 19 isoquinoline derivatives and $MPP^+$ to accumulate and inhibit respiration in intact rat liver mitochondria, assessed using polarographic techniques. None of the compounds examined inhibited respiration supported by either succinate + rotenone or tetramethylparaphenylenediamine (TMPD) + ascorbate. However, with glutamate + malate as substrates, 15 isoquinoline derivatives and $MPP^+$ inhibited state 3 and, to a lesser extent, state 4 respiration in a time-dependent manner. None of the isoquinoline derivatives were more potent than $MPP^+$. 6,7-Dimethoxy-1-styryl-3,4-dihydroisoquinoline uncoupled mitochondrial respiration. Qualitative structure-activity relationship studies revealed that isoquinolinium cations were more active than isoquinolines in inhibiting mitochondrial respiration; these, in turn, were more active than dihydroisoquinolines and 1,2,3,4-tetrahydroisoquinolines. Three-dimensional quantitative structure-activity relationship studies using Comparative Molecular Field Analysis showed that the inhibitory potency of isoquinoline derivatives was determined by steric, rather than electrostatic, properties of the compounds. A hypothetical binding site was identified that may be related to a rate-limiting transport process, rather than to enzyme inhibition. In conclusion, isoquinoline derivatives are less potent in inhibiting respiration in intact mitochondria than impairing complex I activity in mitochondrial fragments. This suggests that isoquinoline derivatives are not accumulated by mitochondria as avidly as $MPP^+$. The activity of charged and neutral isoquinoline derivatives implicates both active and passive processes by which these compounds enter mitochondria, although the quaternary nitrogen moiety of the isoquinolinium cations favours mitochondrial accumulation and inhibition of respiration. These findings suggest that isoquinoline derivatives may exert mitochondrial toxicity *in vivo* similar to that of MPTP/$MPP^+$. BIOCHEM PHARMACOL 51;11: 1503–1511, 1996.

**KEY WORDS.** Parkinson's disease; 1,2,3,4-tetrahydroisoquinoline; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 1-methyl-4-phenylpyridinium; comparative molecular field analysis; 3-dimensional quantitative structure-activity relationship

PD¶ is primarily caused by degeneration of the neuromelanin-containing neurones of the substantia nigra pars compacta (SNc) and the resulting loss of the dopaminergic nigrostriatal pathway. The cause of nigral degeneration is unknown, but postmortem studies suggest that free radical generation and impaired antioxidant defences lead to oxidative stress [1]. These observations relate to findings of increased iron levels, but decreased ferritin content [2], increased mitochondrial superoxide dismutase activity [3] and decreased levels of reduced glutathione [4], leading to increased lipid peroxidation [5]. In addition, there is reduced NADH ubiquinone reductase (complex I) activity and reduced immunostaining for α-KGDH in PD [6–8].

The cause of complex I and α-KGDH defects in PD is unknown. There does not appear to be any significant alteration to the subunits of complex I or their encoding [6–8]. Rather, toxin action is suggested by the ability of MPTP, *via* its active metabolite $MPP^+$, to inhibit complex

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¶ *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; CoMFA, comparative molecular field analysis; MAO-B, monoamine oxidase B; RCR, respiratory control ratio; α-KGDH, α-ketoglutarate dehydrogenase; TMPD, tetramethylparaphenylenediamine; SAR, structure-activity relationship; 3D-QSAR, 3-dimensional quantitative SAR; SNc, substantia nigra pars compacta.

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I and α-KGDH activity, so depleting ATP and inducing cell death [9, 10]. The ability of $MPP^+$ to inhibit mitochondrial function depends on its energy-dependent accumulation within mitochondria, where it reaches millimolar levels [11, 12]. However, immunohistochemical studies have failed to identify MPTP/$MPP^+$-like substances in substantia nigra in PD, so other toxic substances have been sought [13].

Isoquinoline derivatives (e.g. 1,2,3,4-tetrahydroisoquinolines) structurally related to MPTP or $MPP^+$ have emerged as candidate endogenous neurotoxins involved in PD [14]. We [15] and others [16, 17] have demonstrated that such isoquinoline derivatives are also inhibitors of complex I and α-KGDH. Recently, 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) on complexes I, II-III, and IV, and on the nonrespiratory chain enzymes, citrate synthase, glutamate dehydrogenase, and α-KGDH, in rat brain mitochondrial fragments [15, 18]. Several of these compounds were found to be selective inhibitors of complex I and α-KGDH, and some were more potent than $MPP^+$. The lipophilicity of the molecules appeared to be an important factor determining complex I inhibition.

However, these studies did not indicate whether or not isoquinoline derivatives can inhibit mitochondrial function in intact mitochondria, or whether these compounds are concentrated by mitochondria in a similar manner to $MPP^+$. There is some evidence to suggest that this is the case because 1,2,3,4-tetrahydroisoquinoline and its oxidised congener, N-methylisoquinolinium, and the related alkaloids, tetrahydropapaveroline, tetrahydropapaverine, and salsolinol, concentration-dependently inhibit state 3 and state 4 respiration supported by glutamate + malate, pyruvate + malate, or α-ketoglutarate and reduce ATP synthesis in intact mouse brain mitochondria [19, 20]. However, the selectivity, potency, and structural requirements of isoquinoline derivatives for inhibition of respiration in intact mitochondria have not been characterised. This is important because studies with MPTP/$MPP^+$ analogs suggest that a nitrogen moiety positively charged at intracellular pH is required for concentration within mitochondria by an electrochemical gradient, and that lipophilicity is necessary to gain access to the complex I inhibitory site [11, 12, 21].

To determine the ability of isoquinoline derivatives to inhibit respiration in intact mitochondria, we have studied the effects of 19 neutral and quaternary isoquinoline derivatives (11 isoquinolines, 2 dihydroisoquinolines, and 6 1,2,3,4-tetrahydroisoquinolines) on respiration in intact rat liver mitochondria in the presence of various substrates, followed by structure-activity relationship studies to analyse the data.

## MATERIALS AND METHODS

### *Materials*

$MPP^+$ iodide **(w)** was obtained from Research Biochemicals Inc. (Natick, MA). Essentially fatty acid free BSA was obtained from Sigma Chemical Co. (Poole, U.K.). 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–v)** were a gift from Dr. B. Goodwin (King's College London) and were synthesised as previously described [15]. Isoquinoline **(a)**, 1,2,3,4-tetrahydroisoquinoline hydrochloride **(n)**, N-methyl-1,2,3,4-tetrahydroisoquinoline **(o)**, N-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride **(q)** and chemicals used in the synthesis of the other isoquinoline derivatives were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). The other isoquinoline derivatives were prepared as described elsewhere [15]. The chemical structure and purity of the isoquinoline derivatives (Fig. 1) were analysed by NMR, IR, elemental analysis, HPLC, and GC-MS. All other chemicals were of analytical grade and obtained from commercial sources.

### *Isolation of Rat Liver Mitochondria*

Mitochondria were isolated from the liver of male Wistar rats (200–250 g; Bantin & Kingman, Hull, U.K.) by standard differential centrifugation techniques [22]. Rats were killed by stunning and cervical dislocation and the liver rapidly removed and placed on ice. All subsequent procedures were performed at 4°C in isolation medium containing 225 mM mannitol, 75 mM sucrose, 1 mM $K^+$EDTA, 10 mM MOPS-KOH (pH 7.4) and 0.5 mg/mL BSA. Mitochondrial isolates were suspended in isolation medium to a final protein concentration of 10 mg/mL, determined by the folin phenol method using BSA as protein standard [23]. Electron microscopy (data not shown) and biochemical studies revealed that this procedure consistently produced intact and metabolically active mitochondria with respiratory control ratio (RCR; state 3/state 4) of >8 and >5 for 10 mM glutamate + 2.5 mM malate and 10 mM succinate + 100 μM rotenone, respectively [24].

### *Polarographic Determination of Mitochondrial Respiration*

Mitochondrial oxygen consumption was measured polarographically using a Clark-type oxygen electrode (Rank Bros. Ltd., Cambridge, U.K.) in a thermostatically controlled incubation chamber at 25°C containing 3.0 mL of incubation medium (25 mM sucrose, 75 mM mannitol, 100 mM KCl, 0.05 mM $K^+$EDTA, 0.5 mg BSA, 10 mM Tris-phosphate, 10 mM Tris-HCl, pH 7.4) and 0.8 mg mitochondrial protein [25]. Oxygen consumption was supported by using 10 mM glutamate + 2.5 mM malate, 10 mM succinate + 100 μM rotenone or 50 μM TMPD + 2 mM ascorbate as substrates. State 3 respiration was initiated by the addition of 0.25 mM ADP [24].

### *Assessment of the Effects of Isoquinoline Derivatives and $MPP^+$ on Mitochondrial Respiration*

After mitochondria were added to the incubation medium and allowed to equilibrate for 3 min, substrates were added

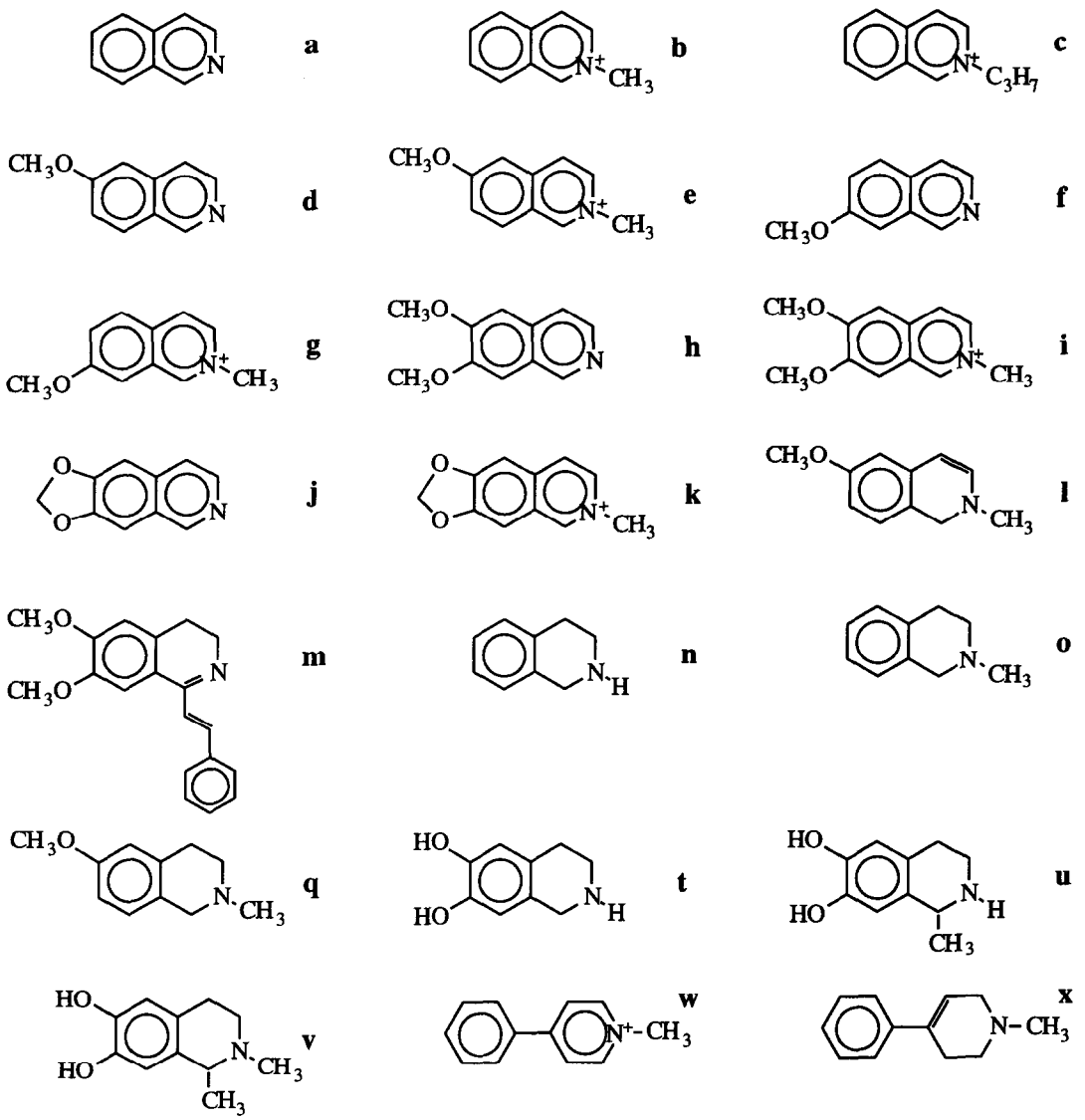


FIG. 1. Structures of the isoquinoline derivatives. **a.** Isoquinoline (IQ); **b.** N-Methylisoquinolinium iodide (N-Me-IQ$^+$); **c.** N-*n*-Propylisoquinolinium iodide (N-Pr-IQ$^+$); **d.** 6-Methoxyisoquinoline (6-OMe-IQ); **e.** N-Methyl-6-methoxyisoquinolinium iodide (N-Me-6-OMe-IQ$^+$); **f.** 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); **q.** N-Methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (N-Me-6-OMe-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$^+$); **x.** 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

to the final concentrations specified above. To determine the time-dependent inhibition of mitochondrial respiration, the isoquinoline derivatives or MPP$^+$ (0.5 mM) were added to the incubation medium with the substrates and allowed to incubate for up to 20 min before state 3 respiration was initiated. The rate of oxygen consumption during state 3 was determined from the linear part of the recording made after the addition of ADP. When most of the added ADP was utilised, the respiratory rate slowed (state 4 respiration) [24]. The rate of oxygen consumption during state 4 was determined at this stage from the linear part of the recording. When state 3 respiration was completely inhibited by some isoquinoline derivatives or MPP$^+$, state 4 respiration was determined from the rate prior to the addi-

tion of ADP. Respiratory rates are expressed as ng atoms O/min/mg.

### *Statistical Analysis*

All respiratory measurements were performed in triplicate and data presented as mean ± SEM. Results were compared using the Mann-Whitney *U*-test.

### *Three-dimensional Quantitative Structure-Activity Relationships (3D-QSAR)*

The starting geometries of the investigated isoquinoline derivatives were constructed using the Sybyl 6.04 software (Tripos Associates, St. Louis, MO, U.S.A.) running on a Silicon Graphics Indigo R4000 and Iris 4D-35 personal workstations [26]. The AM1 Hamiltonian from the MOPAC program was used to minimize the geometry of each molecule. Default settings and AM1 charges were used, except the option "drop-electrostatic," which was set to "No." The grid size had a resolution of 1.5 Å. The Comparative Molecular Field Analysis (CoMFA) study was performed using the ranked order of potency (based on the % inhibition of glutamate + malate supported respiration after 20 min incubation; Table 2) of the isoquinoline derivatives as biological parameter to derive 3D-QSAR models.

## RESULTS

### *Glutamate + Malate-Supported Respiration*

Using glutamate + malate as substrates, 15 isoquinoline derivatives and $MPP^+$ **(w)** inhibited state 3 respiration in a time-dependent manner (Table 1). State 4 respiration was either slightly inhibited or unaffected and the RCR (state 3/state 4) were either reduced or remain unchanged (Table 1). In the isoquinoline series, 6,7-diOMe-IQ **(h)** was inactive and the others inhibited state 3 respiration with varying potencies (Table 2). 6-OMe-IQ **(d)** and N-Me-6,7-diOMe-$IQ^+$ **(i)**, the most and least potent isoquinoline derivatives, respectively, inhibited state 3 respiration by 82.4% and 13.2% ($P < 0.05$) after 5-min incubation (Table 1). 6-OMe-IQ **(d)**, N-Me-6-OMe-$IQ^+$ **(e)** and N-Me-6,7-$OCH_2O$-$IQ^+$ **(k)** completely inhibited state 3 respiration after 20-min incubation, but only N-Me-6,7-$OCH_2O$-$IQ^+$ **(k)** completely inhibited respiration after 10-min incubation (Table 1). Between the two dihydroisoquinolines studied, N-Me-6-OMe-1,2-DHIQ **(l)** was inactive and 6,7-diOMe-1-S-3,4-DHIQ **(m)** uncoupled mitochondrial respiration (Table 1). Norsalsolinol was inactive and THIQ **(n)** inhibited state 3 respiration by 36% ($P < 0.05$) after 5-min incubation (Table 1). The other 1,2,3,4-tetrahydroisoquinolines studied produced small, but statistically significant, inhibitions (8–18%) of state 3 respiration after 20-min incubation (Table 1). None of the isoquinoline derivatives were more potent than $MPP^+$ **(w)**, which inhibited state 3 respiration by 83.6% and 100% ($P < 0.05$) after 5-min, and 10-min incubation, respectively (Table 1).

### *Succinate + Rotenone and TMPD + Ascorbate Supported Respiration*

A subset of six isoquinoline derivatives, IQ **(a)**, N-Me-$IQ^+$ **(b)**, 6,7-$OCH_2O$-IQ **(j)**, 6,7-diOMe-1-S-3,4-DHIQ **(m)**,

**TABLE 1. Effects of isoquinoline derivatives and $MPP^+$ (0.5 mM) on glutamate + malate supported respiration in rat liver mitochondria**

| | State 3 | | | State 4 | | | RCR |
|---|---|---|---|---|---|---|---|
| **Incubation time** | **5** | **10** | **20** | **5** | **10** | **20** | **10** |
| Control | 105.3 ± 3.2 | 105.9 ± 4.9 | 105.4 ± 3.7 | 12.9 ± 1.2 | 12.6 ± 0.2 | 12.8 ± 0.9 | 8.4 |
| IQ **(a)** | 86.7 ± 6.4* | 74.0 ± 4.6* | 72.4 ± 6.3* | 10.1 ± 0.9* | 9.5 ± 1.1* | 9.4 ± 0.5* | 7.8 |
| N-Me-$IQ^+$ **(b)** | 70.0 ± 6.6* | 36.0 ± 2.6* | 30.2 ± 3.1* | 11.5 ± 1.2 | 10.8 ± 0.1* | 10.8 ± 0.7* | 3.3 |
| N-Pr-$IQ^+$ **(c)** | 64.8 ± 5.3* | 39.5 ± 5.5* | 22.5 ± 3.2* | 13.5 ± 2.3 | 11.8 ± 1.6 | 11.0 ± 1.2 | 3.3 |
| 6-OMe-IQ **(d)** | 18.7 ± 1.8* | 9.7 ± 0.5* | 0.0 ± 0.0* | 7.6 ± 0.6* | 9.1 ± 1.6* | 7.2 ± 1.5* | 1.1 |
| N-Me-6-OMe-$IQ^+$ **(e)** | 73.3 ± 5.1* | 25.2 ± 2.0* | 0.0 ± 0.0* | 12.3 ± 0.4 | 8.5 ± 1.1* | 8.9 ± 1.9* | 3.0 |
| 7-OMe-IQ **(f)** | 54.8 ± 4.1* | 54.5 ± 4.7* | 54.3 ± 6.1* | 10.6 ± 1.4 | 10.6 ± 0.6 | 11.9 ± 1.8 | 5.2 |
| N-Me-7-OMe-$IQ^+$ **(g)** | 84.6 ± 3.4* | 34.8 ± 2.3* | 26.7 ± 3.6* | 14.1 ± 1.2 | 10.9 ± 1.0 | 11.1 ± 1.2 | 3.2 |
| 6,7-diOMe-IQ **(h)** | 111.6 ± 22.6 | 119.0 ± 23.8 | 108.4 ± 11.4 | 15.3 ± 1.7 | 15.3 ± 1.4 | 13.2 ± 0.5 | 7.8 |
| N-Me-6,7-diOMe-$IQ^+$ **(i)** | 91.4 ± 6.0* | 91.0 ± 3.2* | 91.3 ± 9.5* | 11.9 ± 1.3 | 12.2 ± 1.6 | 11.4 ± 1.1 | 7.5 |
| 6,7-$OCH_2O$-IQ **(j)** | 51.1 ± 5.2* | 56.1 ± 1.7* | 51.2 ± 1.6* | 12.0 ± 0.5 | 13.3 ± 1.4 | 14.0 ± 1.6 | 4.2 |
| N-Me-6,7-$OCH_2O$-$IQ^+$ **(k)** | 45.7 ± 6.7* | 0* | 0.0 ± 0.0* | 9.8 ± 1.0* | 9.6 ± 1.0* | 9.8 ± 2.0* | 0 |
| N-Me-6-OMe-1,2-DHIQ **(l)** | 106.9 ± 4.7 | 108.0 ± 38.3 | 108.0 ± 4.7 | 12.2 ± 5.2 | 11.6 ± 2.8 | 14.7 ± 2.8 | 9.3 |
| 6,7-diOMe-1-S-3,4-DHIQ **(m)** | UC | UC | UC | 19.6 + 4.6* | 22.4 ± 3.5* | 22.2 ± 5.5* | – |
| THIQ **(n)** | 67.6 ± 3.4* | 65.0 ± 2.4* | 59.9 ± 5.4* | 9.1 ± 0.7* | 8.3 ± 0.5* | 8.2 ± 0.6* | 7.8 |
| N-Me-THIQ **(o)** | 114.4 ± 6.8 | 103.4 ± 6.7 | 86.8 ± 3.5* | 15.0 ± 0.9 | 15.0 ± 1.4 | 15.1 ± 1.2 | 6.9 |
| N-Me-6-OMe-THIQ **(q)** | 112.8 ± 6.1 | 107.9 ± 6.6 | 95.4 ± 6.6* | 12.7 ± 1.5 | 11.9 ± 0.9 | 12.4 ± 1.0 | 9.1 |
| Norsalsolinol **(t)** | 100.9 ± 5.4 | 104.3 ± 1.0 | 108.9 ± 4.0 | 13.3 ± 1.1 | 13.3 ± 1.1 | 12.8 ± 2.0 | 7.8 |
| Salsolinol **(u)** | 111.7 ± 6.4 | 101.9 ± 3.4 | 92.2 ± 3.4* | 14.0 ± 1.3 | 11.7 ± 0.4 | 12.1 ± 0.8 | 8.7 |
| N-Me-salsolinol **(v)** | 102.9 ± 5.4 | 100.9 ± 3.3 | 91.7 ± 2.3* | 11.2 ± 0.3 | 11.5 ± 1.8 | 11.0 ± 1.4 | 8.8 |
| $MPP^+$ **(w)** | 17.3 ± 1.1* | 0* | 0* | 9.7 ± 0.6* | 9.7 ± 0.4* | 9.8 ± 0.7* | 0 |

Rat liver mitochondria (0.8 mg) in the presence of 10 mM glutamate + 2.5 mM malate, were incubated at 25°C with each compound (0.5 mM) under investigation for 5, 10, or 20 min before state 3 respiration was initiated by the addition of 0.25 mM ADP. All assays were performed in triplicate and data presented as mean ± SEM. Respiratory rates are expressed as ng atoms O/min/mg. *$P < 0.05$ (Mann-Whitney *U*-test). RCRs (state 3/state 4) were calculated at 10 min. UC, uncoupled.

**TABLE 2. Ranked order of potency of the isoquinoline derivatives and $MPP^+$ towards inhibition of mitochondrial respiration and complex I activity**

| Compound | Ranked order of potency | |
|---|---|---|
| | Inhibition of State 3 respiration | Inhibition of Complex I activity |
| $MPP^+$ (w) | 100 | 67.8 |
| 6-OMe-IQ (d) | 100 | 100 |
| N-Me-6,7-$OCH_2O$-$IQ^+$ (k) | 100 | 33.9 |
| N-Me-6-OMe-$IQ^+$ (e) | 100 | 100 |
| N-Pr-$IQ^+$ (c) | 78.7 | 0 |
| N-Me-7-OMe-$IQ^+$ (g) | 74.7 | 100 |
| N-Me-$IQ^+$ (b) | 71.4 | 100 |
| 6,7-$OCH_2O$-IQ (j) | 51.4 | 100 |
| 7-OMe-IQ (f) | 48.5 | 100 |
| THIQ (n) | 43.2 | 18.9 |
| IQ (a) | 31.3 | 100 |
| N-Me-THIQ (o) | 17.6 | 64.1 |
| N-Me-6,7-diOMe-$IQ^+$ (i) | 13.4 | 100 |
| N-Me-salsolinol (v) | 13 | 58.0 |
| Salsolinol (u) | 12.5 | 55.9 |
| N-Me-6-OMe-THIQ (q) | 9.5 | 100 |
| Norsalsolinol (t) | 0 | 6.0 |
| 6,7-diOMe-IQ (h) | 0 | 39.9 |
| N-Me-6-OMe-1,2-DHIQ (l) | 0 | 100 |

The column, Inhibition of State 3 respiration, presents the compounds in decreasing order of potency according to the percent inhibition of state 3 respiration supported by glutamate + malate after 20-min incubation and at a concentration of 0.5 mM. The column, Inhibition of Complex I activity, presents the percent inhibition of complex I activity in rat brain mitochondrial fragments by isoquinoline derivatives and $MPP^+$ at a concentration of 10 mM. Data obtained from [15].

THIQ (n), N-Me-THIQ (o) and $MPP^+$ (w) were chosen to reflect the structural diversity, respiratory activity (inhibitor/uncoupler), and potencies of the 19 isoquinoline derivatives studied for their effects on glutamate + malate-supported respiration. The effects of these compounds on succinate + rotenone- and TMPD + ascorbate-supported respiration were investigated. Neither the isoquinoline derivatives nor $MPP^+$ (w) (10-min incubation) inhibited respiration supported by these substrates (Table 3). 6,7-diOMe-1-S-3,4-DHIQ (m) uncoupled mitochondrial respiration supported by succinate + rotenone (Table 3).

### *Structure-Activity Relationships*

Qualitative structure-activity relationship studies revealed that isoquinolinium cations were more active than isoquinolines in inhibiting mitochondrial respiration which, in turn, were more active than dihydroisoquinolines and 1,2,3,4-tetrahydroisoquinolines. The influence of structural motifs on the activity of the isoquinoline derivatives were examined by comparing, pairwise, each compound with each other. Some rationalisations proved possible, but such influences could only be defined qualitatively as modest (+ or −) or marked (++ or −−) because the effect of most substituents varied from one compound to the other (Table 4). Two 3D-QSAR models were constructed based on the steric (A1) and electrostatic (A2) field and a third model, based on a combination of both fields (A3) (Table 5). Model A2 based on the electrostatic field alone was invalid (cross-validated correlation coefficient = 0.05) but models based on the steric field (A1, A3) were of good statistical quality. However, the cross-validated correlation coefficient of model A3 was somewhat below that of model A1, and the contribution of the electrostatic field in model A3 was marginal (13%) relative to that of the steric field (87%). The best model is, thus, A1, which expresses only a steric contribution and is depicted graphically in Fig. 2 and Fig. 3.

## DISCUSSION

The cascade of events leading to destruction of the SNc in PD is unclear, but studies of postmortem brain tissue from patients dying with PD have identified defects in complex I and α-KGDH suggesting that failure of mitochondrial energy metabolism may contribute to the neurodegenerative process [1, 6, 7]. Isoquinoline derivatives are potent inhibitors of complex I and α-KGDH activity in mitochondrial fragments, but little is known of their effects on respiration in intact mitochondria, and the structural requirements for inhibition have not previously been investigated. Previous studies with MPTP/$MPP^+$ analogs have shown the importance of a quaternary nitrogen moiety [11, 12, 21]. Indeed, some MPTP/$MPP^+$ analogs, such as 4-phenylpyridines, that are potent inhibitors of complex I in mitochondrial fragments, are without effect on intact mitochondria because they do not possess the necessary steric/electrostatic properties for mitochondrial accumulation [27]. Conversely, some MPTP/$MPP^+$ analogs that are weak inhibitors of complex I in mitochondrial fragments, such as N-methylpyridinium cations including $MPP^+$ (w), become potent inhibitors of respiration due to their active mitochondrial concentration [27].

We, now, show that isoquinoline derivatives are time-dependent inhibitors of glutamate + malate-supported respiration in intact rat liver mitochondria. Previous to this study, only a few isoquinoline derivatives including IQ (a) and salsolinol (u), have been studied for their effects on mitochondrial respiration. The potencies of these compounds in this study are in good agreement with those previously reported. For example, in this study, salsolinol (u) was found to be essentially inactive as previously reported [20], and the concentration of IQ (a) that inhibited state 3 respiration by 50% was 0.5 mM, in agreement with 0.31 mM reported elsewhere [19]. Similarly, in this study, $MPP^+$ (w) inhibited state 3 respiration after 5-min and 10-min incubation by 83.6% and 100%, respectively, comparable to previous reports of approximately 90% and 100%. None of the isoquinoline derivatives were, however, more potent than $MPP^+$.

The metabolism of glutamate by glutamate dehydroge-

**TABLE 3. Effects of isoquinoline derivatives and MPP$^+$ (0.5 mM) on respiration supported by succinate + rotenone or TMPD + ascorbate in rat liver mitochondria**

| Compound | Respiratory parameter | Substrates | |
|---|---|---|---|
| | | Succinate + Rotenone | TMPD + Ascorbate |
| Control | State 3 | 149.6 ± 7.3 | 44.9 ± 2.7 |
| | State 4 | 27.7 ± 1.2 | 34.1 ± 1.2 |
| | RCR | 5.4 | 1.3 |
| IQ (a) | State 3 | 141.1 ± 16.3 | 44.7 ± 1.2 |
| | State 4 | 27.6 ± 2.8 | 35.2 ± 3.6 |
| | RCR | 5.1 | 1.3 |
| N-Me-IQ$^+$ (b) | State 3 | 148.9 ± 0.4 | 42.9 ± 5.7 |
| | State 4 | 28.0 ± 1.4 | 34.7 ± 2.4 |
| | RCR | 5.3 | 1.2 |
| 6,7-$OCH_2O$-IQ (j) | State 3 | 141.4 ± 17.6 | 50.2 ± 7.3 |
| | State 4 | 27.3 ± 1.9 | 35.2 ± 1.2 |
| | RCR | 5.2 | 1.4 |
| 6,7-diOMe-1-S-3,4-DHIQ (m) | State 3 | UC | |
| | State 4 | 76.0 ± 10.0* | ND |
| | RCR | UC | |
| THIQ (n) | State 3 | 138.2 ± 10.8 | 42.7 ± 6.0 |
| | State 4 | 24.9 ± 1.9 | 36.1 ± 4.2 |
| | RCR | 5.6 | 1.2 |
| N-Me-THIQ (o) | State 3 | 151.8 ± 12.7 | 40.8 ± 5.2 |
| | State 4 | 28.4 ± 2.1 | 35.2 ± 3.7 |
| | RCR | 5.3 | 1.2 |
| MPP$^+$ (w) | State 3 | 141.1 ± 4.5 | 43.1 ± 4.2 |
| | State 4 | 29.8 ± 3.7 | 35.2 ± 1.8 |
| | RCR | 4.7 | 1.2 |

Rat liver mitochondria (0.8 mg), in the presence of 20 mM succinate + 100 μM rotenone or 50 μM TMPD + 2 mM ascorbate, were incubated at 25°C with each compound (0.5 mM) under investigation for 10 min before state 3 respiration was initiated by the addition of 0.25 mM ADP. All assays were performed in triplicate and data presented as mean ± SEM. Respiratory rates are expressed ng atoms O/min/mg. *$P < 0.05$ (Mann-Whitney *U*-test). RCR (state3/state4). ND, Not Determined; UC, Uncoupled.

nase provides reducing equivalents to the respiratory chain *via* complex I. Thus, inhibition of glutamate + malate-supported respiration by isoquinoline derivatives and MPP$^+$ (w) suggests inhibition of electron transfer at the level of complex I because the activity of glutamate dehydrogenase is not affected by these compounds [10, 18]. Although aspartate aminotransferase and malate dehydrogenase are involved in glutamate + malate-mediated electron transfer, inhibition of these enzymes does not appear to contribute to the impairment of mitochondrial respiration because isoquinoline derivatives, such as norsalsolinol (t), that are not inhibitors of complex I activity in mitochondrial fragments [15] are also unable to inhibit glutamate + malate-supported respiration. We also found that some isoquinoline derivatives, IQ (a), N-Me-IQ$^+$ (b), N-Me-6,7-diOMe-IQ$^+$ (i), and N-Me-6-OMe-THIQ (q), for example, which are markedly more potent than MPP$^+$ in inhibiting com-

**TABLE 4. Structural motifs influencing the inhibition of mitochondrial respiration by isoquinoline derivatives**

| | |
|---|---|
| A) Structural motifs favourable to activity: | |
| • 2-Me+ | (+)* |
| • 2-Pr+ | (+) |
| • 6-OMe | (+) |
| • 7-OMe | (+) |
| • 6,7-$OCH_2O$ | (++)† |
| B) Structural motifs unfavourable to activity: | |
| • 2-Me | (−)‡ |
| • 6,7-diOMe | (−−)§ |
| • 6,7-diOH | (−−) |
| • 1,2,3,4-tetrahydro | (−−) |
| • 1,2-dihydro | (−−) |
| C) Structural motifs without detectable influence on activity: | |
| • 1-Me | (0) |

* Modest favourable influence; † marked favourable influence; ‡ modest unfavourable influence; § marked unfavourable influence.

**TABLE 5. Statistical results of a CoMFA study of the effects of isoquinoline derivatives on mitochondrial respiration**

| Model | Field | $q^2$ | N | $r^2$ | Relative contribution | |
|---|---|---|---|---|---|---|
| | | | | | ste | ele |
| A1 | steric field (ste) | 0.66 | 2 | 0.86 | 1 | – |
| A2 | electrostatic field (ele) | 0.05 | 2 | – | – | – |
| A3 | ste and ele | 0.64 | 3 | 0.87 | 0.87 | 0.13 |

$q^2$, cross-validated correlation coefficient; N, optimal number of principal components used for the final analysis; $r^2$, squared correlation coefficient.

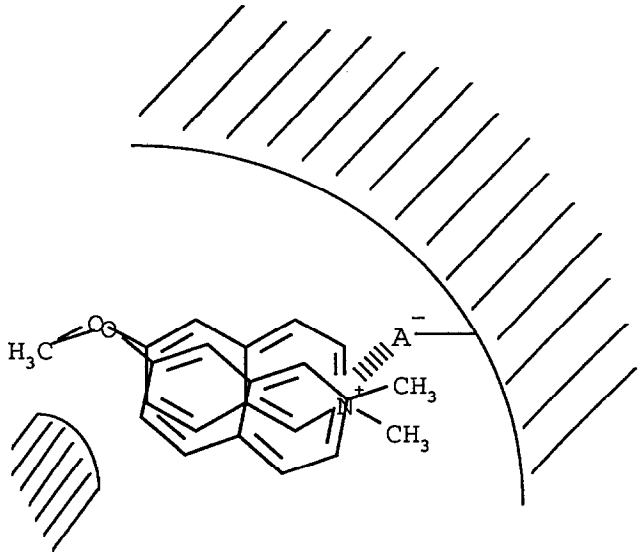


**FIG. 2. Proposed hypothetical binding site of isoquinoline derivatives. Compounds e and g are positioned in the binding site according to the alignment of model A1. The flat binding site accommodates its ligands on either of their sides and anchors them with an ionic bond ($A^-$–$N^+$) and van der Waals interactions. The marked difference in activity between dimethoxy- or dihydroxy-substituted compounds on the one hand, and monomethoxy- or methylenedioxy-substituted compounds on the other hand, can be explained by steric hindrance.**

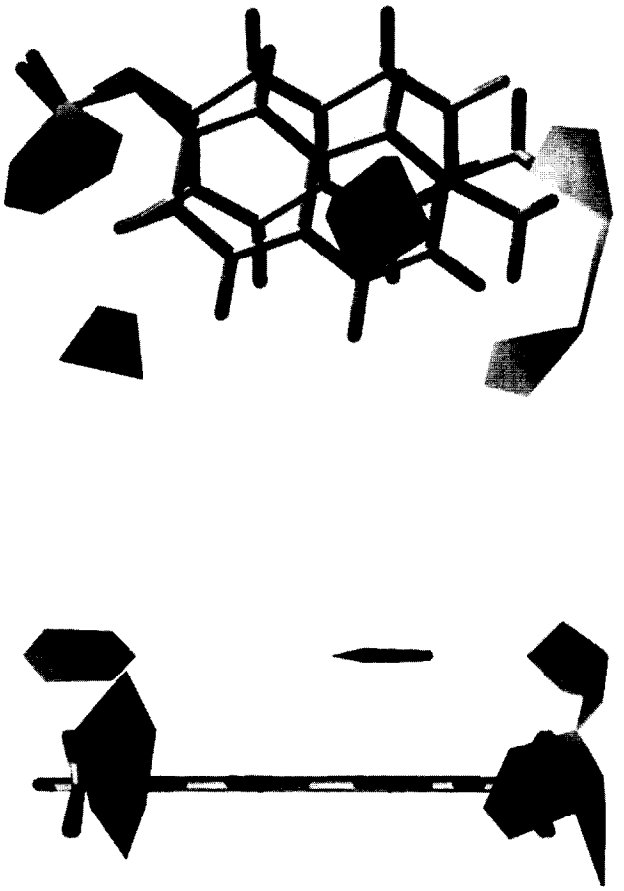

**FIG. 3. Graphical representation of model A1 rationalizing the inhibition of mitochondrial respiration by the isoquinoline derivatives. The greyscale code is as follows: sterically favourable zones are in light grey while sterically unfavourable zones are in dark grey. The contour of field distribution is 15% in dark grey and 75% in light grey. The view is from above (upper part of the Figure) and from the side (lower part of the Figure), with compounds e and g in place. A substituent on the nitrogen (position 2) is shown to point in a sterically favourable zone, while a bulky substituent in position 7 would be unfavourable. A single methoxy group or a methylenedioxy group can make a favourable contribution, whereas a saturated ring system makes an unfavourable contribution.**

plex I activity in mitochondrial fragments [15], are less effective inhibitors of glutamate + malate-supported mitochondrial respiration. These findings suggest that isoquinoline derivatives are not accumulated by mitochondria as avidly as $MPP^+$ (**w**).

None of the isoquinoline derivatives or $MPP^+$ (**w**) inhibited respiration supported by either succinate + rotenone or TMPD + ascorbate, which supply reducing equivalents to the respiratory chain *via* complex II and complex IV, respectively. This suggests that these compounds are not inhibitors of these enzymes, and is in agreement with previous reports on the inability of MPTP, $MPP^+$ and close pyridine analogs, and isoquinoline derivatives, to inhibit mitochondrial respiration supported by succinate + rotenone and TMPD + ascorbate or complex II and complex IV activity in mitochondrial fragments [15–17, 20, 28, 29].

The dihydroisoquinoline, 6,7-diOMe-1-S-3,4-DHIQ (**m**), was an uncoupler, rather than an inhibitor, of mitochondrial respiration. This appears to be a consequence of the styryl substituent because the other dihydroisoquinoline examined, N-Mc-6-OMe-1,2-DHIQ (**l**), was inactive. Indeed, some β-carbolines structurally related to MPTP/$MPP^+$ and isoquinoline derivatives are uncouplers of mitochondrial respiration [19].

The order of potency of the isoquinoline derivatives (isoquinolinium cations > isoquinolines > dihydroisoquinolines and 1,2,3,4-tetrahydroisoquinolines), as revealed by qualitative SAR, agrees with previous studies showing that pyridinium cations are more potent than their corresponding neutral congeners in inhibiting mitochondrial respiration [21]. This suggests that the quaternary nitrogen moiety of the isoquinolinium cations and active mitochondrial transport processes are important factors influencing the inhibition of mitochondrial respiration by isoquinoline derivatives. However, the activity of neutral isoquinoline derivatives also implicates passive membrane transport in the mitochondrial accumulation of some of these compounds. The influence of structural motifs on the activity of isoquinoline derivatives could only be defined qualitatively as modest or marked because the effects of most substituents vary from one compound to the other. Thus, a monomethoxy group in either the 6- or 7-position appears to be favourable to activity, and a 6,7-dimethoxy or a 6,7-dihydroxy substitution is highly unfavourable.

To gain some insights into the pharmacophore, the inhibitory activity of the isoquinoline derivatives were subjected to CoMFA. All molecules, except the aromatic isoquinoline derivatives, were constructed in their protonated form because THIQ derivatives, having pKa values of approximately 9, are protonated at physiological pH. The minimal energy conformation of all compounds were calculated by the semiempirical AM1 method and then superimposed. The superimposition in a biologically relevant conformation is a critical step in any CoMFA study, and particularly in this analysis. Indeed, a preliminary analysis using the most obvious superimposition of the ring system yielded an invalid model (cross-validated correlation coefficient < 0.4; data not shown). A simple comparison of structures and data showed that monomethoxylated compounds were active irrespective of the 6- or 7-position of the group, whereas 6,7-dimethoxy derivatives were always inactive. This led to the conclusion that a more realistic model might be that shown in Fig. 2. This mode of superimposition was chosen for all compounds and the resulting models summarised in Table 5. The best model, **A1,** which expresses only a steric contribution, is represented graphically in Fig. 3, showing the steric influence of substituents, namely the regions where a substituent has a favourable (light grey) or unfavourable (dark grey) steric influence. Figure 3 also shows that a substituent on the nitrogen atom (position 2), a 6- or 7-monomethoxy substituent, and a 6,7-methylenedioxy moiety are sterically favourable, and a 6,7-dimethoxy substitution is sterically unfavourable (compounds **h, i, t, u**). The sterically unfavourable region above ring B indicates a steric hindrance of out-of-plane atoms in the saturated ring system of THIQ derivatives. Thus, model **A1** validates the hypothesis depicted in Fig. 2. We conclude that the compounds might act by interacting with a hypothetical binding site believed to be a flat pocket, accommodating its ligands on either of their faces and anchoring them with two weak interactions, an ionic bond involving the cationic nitrogen atom, and van der Waals/charge transfer interactions involving the region of electron delocalisation (aromatic ring plus oxygen atoms).

The nature of such a binding site is unknown at present. One logical hypothesis would be that the 3D-QSAR model devised relates to the site and mechanism of action of isoquinoline derivatives within mitochondria, such as the inhibition of an enzyme system critical for mitochondrial respiration. Such a hypothesis is, however, unlikely because no QSAR model could be established for inhibition of complex I and α-KGDH by the same compounds in mitochondrial fragments [15, 18]. Alternatively, the model depicted in Fig. 2 and Fig. 3 is, in fact, that of a membrane transport system that allows influx of the compounds into mitochondria. Thus, we are led at this stage to postulate that the observed differences in the inhibition of respiration in intact mitochondria are primarily due to the rate-limiting transport process, rather than by interactions with enzyme inhibitory sites. Further studies are necessary to clarify this.

In summary, the isoquinoline derivatives selectively inhibited glutamate + malate supported respiration, but less potently than $MPP^+$. Isoquinoline derivatives are less potent in inhibiting respiration in intact mitochondria than impairing complex I activity in mitochondrial fragments, suggesting that isoquinoline derivatives are not accumulated by mitochondria as avidly as $MPP^+$. The inhibition of mitochondrial respiration by charged and neutral isoquinoline derivatives implicates both active and passive processes by which these compounds enter mitochondria, although the quaternary nitrogen moiety of the isoquinolinium cations favours mitochondrial accumulation and inhibition of respiration. 3D-QSAR suggest that the inhibitory potency of isoquinoline derivatives is determined by their steric, more than electrostatic, properties. A hypothetical binding site was identified that may be related to the rate-limiting transport process, rather than to enzyme inhibition. These findings suggest that the mitochondrial toxicity of isoquinoline derivatives are similar to that of $MPTP/MPP^+$.

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