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Inhibition of [^{3}H]dopamine Uptake into Striatal Synaptosomes by Isoquinoline Derivatives Structurally Related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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ABSTRACT. Isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 1-methyl-4-phenylpyridinium (MPP^+) may be endogenous neurotoxins causing nigral cell death in Parkinson's disease. These compounds inhibit mitochondrial function but, like MPP^+, require accumulation in dopaminergic neurones *via* the dopamine reuptake system to exert toxicity. We, now, examine the substrate affinity of 14 neutral and quaternary isoquinoline derivatives (7 isoquinolines, 2 dihydroisoquinolines and 5 1,2,3,4-tetrahydroisoquinolines) for the dopamine reuptake system by their ability to inhibit the uptake of [^{3}H]dopamine into rat striatal synaptosomes. Ten isoquinoline derivatives and MPP^+ inhibited [^{3}H]dopamine uptake in a concentration-dependent manner. Only 5 isoquinoline derivatives produced 50% inhibition of [^{3}H]dopamine uptake (IC_{50} = 8.0–50.0 μM), none of which were as potent as MPP^+ (IC_{50} = 0.33 μM). These findings suggest that isoquinoline derivatives are moderate to poor substrates for the dopamine reuptake system and that high concentrations of, or prolonged exposure to, isoquinoline derivatives may be necessary to cause neurodegeneration. BIOCHEM PHARMACOL 52;1:29–34, 1996.

KEY WORDS. Parkinson's disease; 1,2,3,4-tetrahydroisoquinoline; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 1-methyl-4-phenylpyridinium; synaptosomes; structure-activity relationship

The neurodegenerative process underlying destruction of the dopamine-containing neurones of the substantia nigra pars compacta (SNc) in PD[¶] remains unknown. However, it may be related to the mechanism of action of the selective nigral toxin MPTP. In brain, MPTP is metabolised by glial MAO-B to produce its active metabolite MPP^+ [1]. MPP^+ is actively accumulated by dopaminergic neurones *via* the dopamine reuptake system prior to its energy-dependent concentration within mitochondria [2, 3]. MPP^+ induces cell death by selective inhibition of NADH ubiquinone reductase (complex I) and α-KGDH resulting in ATP depletion [4–6].

Isoquinoline derivatives structurally related to MPTP or MPP^+ may be endogenous neurotoxins contributing to cell death in PD. These compounds, for example THIQ, N-methyl-THIQ, and salsolinol, occur naturally in the brain of animals and humans [7–10]. Isoquinoline derivatives are synthesised *in vivo* by Pictet-Spengler condensation of biogenic amines with aldehydes. They are metabolised by N-methyltransferase and MAO-B to produce N-methylated and quaternary derivatives, such as N-methylisoquinolinium [11–13]. Isoquinoline derivatives are also present in many plants, foodstuffs (e.g. cocoa, milk, and bananas) and alcoholic beverages [14, 15]. Because many of these compounds are able to cross the blood-brain barrier, they may also be considered as potential environmental neurotoxins [16].

Like MPP^+, isoquinoline derivatives are potent inhibitors of complex I and α-KGDH in mitochondrial fragments and, to a lesser extent, inhibit glutamate + malate (but not

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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; α-KGDH, α-ketoglutarate dehydrogenase; SAR, structure-activity relationship; QSAR, quantitative SAR; 3D-QSAR, 3-dimensional QSAR; SNc, substantia nigra pars compacta.

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succinate)-supported respiration in intact mitochondria [17–22]. This suggests that these compounds may also be dopaminergic toxins but, like MPP^+, they must be accumulated by dopamine-containing neurones *via* the dopamine reuptake system. There is some evidence to suggest that this does occur. For example, tritiated 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and 1-methyl-4,6,7-trihydroxy-THIQ are accumulated in rat brain synaptosomes in a concentration-dependent manner [23]. These compounds and salsolinol also inhibit [^{3}H]dopamine uptake in a concentration-dependent manner and, additionally, salsolinol causes release of stored [^{3}H]dopamine [23]. Similarly, N-methylisoquinolinium is accumulated by rat striatal slices and 2-methyl-4,6,7-trihydroxy-THIQ depletes striatal dopamine in rat brain [24, 25]. These actions may reflect functional activity because N-methylisoquinolinium and THIQ are toxic to tyrosine hydroxylase immunoreactive mesencephalic cells in culture, and to nigral neurones on direct application *in vivo* [20, 26–28].

However, the substrate affinity of isoquinoline derivatives for the dopamine reuptake system has not been determined and, unlike MPTP/MPP^+ analogs, the structural characteristics of isoquinoline derivatives influencing uptake is not known. Consequently, we have studied the effects of 14 neutral and quaternary compounds from 3 classes of isoquinoline derivatives (7 isoquinolines, 2 dihydroisoquinolines, and 5 THIQs) on the uptake of [^{3}H]dopamine into rat striatal synaptosomes.

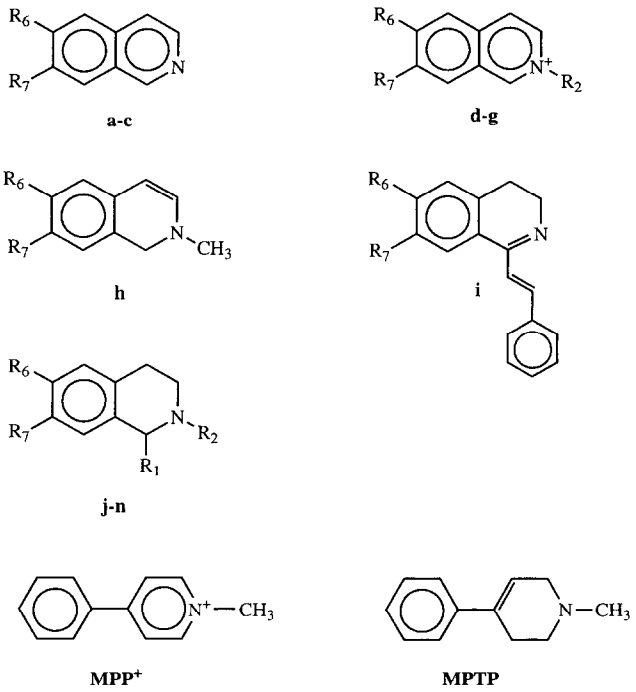

FIG. 1. Structures of the isoquinoline derivatives, MPP^+ and MPTP. See Table 1 for substituent groups.

MATERIALS & METHODS

Materials

[^{3}H]Dopamine (22.15 Ci/mmol) was obtained from New England Nuclear (Du Pont U.K. Ltd). MPP^+ iodide was obtained from Research Biochemicals Inc. (U.S.A.). 6,7-Dimethoxy-1-styryl-3,4-dihydroisoquinoline hydrochloride **(i)** was obtained as a gift from Prof. H. D. Höltje (Freie Universität, Berlin, Germany). The salsolinols **(l-n)** were a gift from Dr. B. Goodwin (King's College, London, U.K.). Isoquinoline **(a)**, THIQ hydrochloride **(j)**, N-methyl-THIQ **(k)**, 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 reported elsewhere [17]. The chemical structure and purity of the isoquinoline derivatives (Fig. 1 and Table 1) were analysed by nuclear magnetic resonance and infrared spectroscopy, elemental analysis, HPLC, and GC-MS. All other chemicals were of analytical grade and obtained from commercial sources.

Preparation of Striatal Synaptosomes

Striatal synaptosomes were prepared from male Wistar rats (200–250 g; Bantin and Kingman, Hull, U.K.) as previously described [2]. Briefly, rats were killed by stunning and cervical dislocation, the brains immediately removed and the striata rapidly dissected out on ice according to anatomical demarcations described elsewhere [29]. The striatal tissue from both hemispheres were combined and homogenised in 0.32 M sucrose (10% w/v) using a Potter-type tissue grinder with 12 up-and-down strokes of a motor-driven (800 rpm) PTFE pestle (total clearance = 0.25 mm). The homogenate was centrifuged at 1000 *g* for 10 min at 4°C in a Sorvall RC-5B refrigerated centrifuge. The supernatant was collected and centrifuged at 12,000 *g* for 20 min at 4°C. The final pelleted synaptosomal fraction was suspended (10% w/v) in modified Krebs medium (120 mM NaCl, 5 mM KCl, 11 mM glucose, 50 mM Tris-HCl, pH 7.4).

[^{3}H]Dopamine uptake

Studies on the uptake of [^{3}H]dopamine into striatal synaptosomes were conducted at 37°C in 1.0 mL of modified Krebs medium containing 1.7 mM ascorbic acid (to prevent auto-oxidation of dopamine), 80 μM pargyline (to block metabolism of dopamine by monoamine oxidase) and 10–1000 nM [^{3}H]dopamine, as previously described [2]. The reaction was initiated by the addition of freshly prepared synaptosomes (0.9 mg striatal tissue based on original wet weight) previously equilibrated at 37°C. Samples were incubated for up to 30 min, then terminated by rapid vacuum filtration through Whatman GF/B 2.1 cm glass-fibre filters (Fisons, U.K.) followed by 2 consecutive washes of the filters with 2.0 mL ice-cold incubation medium. The filters were immediately transferred to scintillation vials containing 5 mL of Optiphase HiSafe II scintillation cocktail (LKB Scintillation Products, U.K.) and equilibrated for 1 hr before the radioactivity (counts per minute) collected on the

TABLE 1. Structures* and [^{3}H]dopamine uptake inhibitory activities of the isoquinoline derivatives

Compound	R_1	R_2	R_6	R_7	IC_{50} (mM)	% inhibition of [^{3}H]dopamine uptake
a	-H	-H	-H	-H		36.0 ± 0.9†
b	-H	-H	-H	$-OCH_3$	10.0	50.0 ± 6.5‡
c	-H	-H	$-OCH_2O-$		NA	37.0 ± 2.4†
d	-H	$-CH_3$	-H	-H	NA	3.3 ± 0.3
e	-H	$-C_3H_7$	-H	-H	NA	32.8 ± 5.6†
f	-H	$-CH_3$	-H	$-OCH_3$	NA	7.0 ± 1.0
g	-H	$-CH_3$	$-OCH_2O-$		NA	7.0 ± 0.9
h	-H	$-CH_3$	$-OCH_3$	-H	10.0	52.4 ± 9.8‡
i	-styryl	-H	$-OCH_3$	$-OCH_3$	NA	6.6 ± 0.9
j	-H	-H	-H	-H	50.0	50.0 ± 7.3‡
k	-H	$-CH_3$	-H	-H	8.0	61.1 ± 7.3‡
l	-H	-H	-OH	-OH	NA	23.5 ± 1.4†
m	$-CH_3$	-H	-OH	-OH	NA	39.3 ± 8.5‡
n	$-CH_3$	$-CH_3$	-OH	-OH	37.0	72.7 ± 1.1‡
MPP^+					0.33	100

The isoquinoline derivatives (100 μM) were added to the incubation medium with 10 nM [^{3}H]dopamine and allowed to equilibrate at 37°C before uptake was initiated by the addition of freshly prepared synaptosomes (0.9 mg striatal tissue). The reaction was terminated after 10 min incubation. Data are presented as mean ± SEM (n = 6). NA, Not attained (these compounds failed to produce 50% inhibition of [^{3}H]dopamine uptake). * see Fig. 1; † $P < 0.05$; ‡ $P < 0.01$ (Student's t-test). Logarithmic extrapolation of the MPP^+ curve indicated that MPP^+ inhibited [^{3}H]dopamine uptake by 100% at 100 μM.

filters was measured by liquid scintillation spectrometry using a Packard Tricarb-460C spectrometer. Nonspecific uptake was determined in parallel experiments conducted on ice at 0–4°C. Active uptake (referred to simply as uptake) is defined as the total uptake (37°C) minus nonspecific uptake (0–4°C). The latter accounted for 6–7% of total uptake. The rate of [^{3}H]dopamine uptake was expressed as nmol/min/g striatal tissue.

Kinetics of [^{3}H]dopamine Uptake

To determine the time-dependency of [^{3}H]dopamine uptake into striatal synaptosomes, incubations were carried out for 5, 10, 20, and 30 min in the presence of 10 nM [^{3}H]dopamine. In experiments to determine the concentration-dependent uptake of [^{3}H]dopamine into striatal synaptosomes, [^{3}H]dopamine (10–1000 nM) was added to the incubation medium and allowed to equilibrate at 37°C before uptake was initiated by the addition of freshly prepared synaptosomes (0.9 mg striatal tissue). The reaction was terminated after a 10-min incubation. Plots of [^{3}H]dopamine concentration vs rate of [^{3}H]dopamine uptake were constructed from which Lineweaver-Burk plots (1/[V] vs 1/[S]) were derived, and K_m and V_{max} determined.

Effects of Isoquinoline Derivatives and MPP^+ on [^{3}H]dopamine Uptake

To determine the effects of the isoquinoline derivatives (1–100 μM) or MPP^+ (0.25–2.0 μM) on the uptake of [^{3}H]dopamine by striatal synaptosomes, the compounds were added to the incubation medium with 10 nM [^{3}H]dopamine and allowed to equilibrate at 37°C. Uptake was initiated by the addition of freshly prepared synaptosomes (0.9 mg striatal tissue) and the reaction terminated after a 10-min incubation.

Structure-activity Relationships

QSAR and 3D-QSAR using CoMFA were performed as previously described [17]. The CoMFA study was performed using the percent inhibition of striatal synaptosomal uptake of [^{3}H]dopamine uptake by the isoquinoline derivatives (100 μM) as the biological parameter (Table 1).

Statistical Analysis

Data were analysed statistically using the Student's t-test.

RESULTS

Kinetics of [^{3}H]dopamine Uptake

Uptake was concentration-dependent and saturable, displaying Michaelis-Menten characteristics with a mean half-maximal rate (K_m) at 0.26 μM [^{3}H]dopamine and a mean maximal rate (V_{max}) of 2.02 nmol/min/g striatal tissue.

Effects of Isoquinoline Derivatives and MPP^+ on [^{3}H]dopamine Uptake

Ten isoquinoline derivatives and MPP^+ inhibited the uptake of [^{3}H]dopamine into striatal synaptosomes in a concentration-dependent manner ($P < 0.05$), but only 5 isoquinoline derivatives and MPP^+ produced 50% of more inhibition. N-Methyl-1,2,3,4-tetrahydroisoquinoline **(k)** (IC_{50} = 8.0 μM), N-methyl-6-methoxy-1,2-dihydroisoquinoline **(h)** (IC_{50} = 10.0 μM), 7-methoxyisoquinoline **(b)** (IC_{50} = 10.0 μM), N-methylsalsolinol **(n)** (IC_{50} = 37.0

μM) and THIQ **(j)** (IC_{50} = 50.0 μM) produced 50% inhibition of [^{3}H]dopamine uptake. None of the isoquinoline derivatives studied were as potent as MPP^+ (IC_{50} = 0.33 μM). The active isoquinoline derivatives inhibited [^{3}H]dopamine uptake by 33–73% ($P < 0.05$) at a concentration of 100 μM (Table 1).

Structure-activity Relationships

The data presented in Table 1 were used in an attempt to derive structure-activity relationships. Although no statistically acceptable QSAR or 3D-QSAR model was found, the favourable or unfavourable influence of some functional groups became apparent when comparing pairs of compounds. THIQs **(j–n)** were more potent than dihydroisoquinolines **(h,i)** or isoquinolines **(a–g)** in inhibiting [^{3}H]dopamine uptake. Isoquinolinium cations **(d–g)** were less active than isoquinolines **(a–c)**. A 2-methyl group (in THIQs) or a 7-methoxy group increased activity, whereas two -OH substituents in positions 6- and 7- were notably unfavourable for activity.

DISCUSSION

The mechanism of action of MPTP continues to provide insight into events leading to destruction of the nigrostriatal pathway in PD. Indeed, the observation of defects of complex I and α-KGDH in the substantia nigra in PD reflects the actions of MPTP/MPP^+ [30, 31]. The identification of isoquinoline derivatives structurally related to MPTP and MPP^+, and their ability to act as inhibitors of complex I and α-KGDH in mitochondrial fragments and intact mitochondria, support the hypothesis that such compounds may be endogenous neurotoxins. Active uptake of MPP^+ into dopaminergic neurones *via* the dopamine reuptake system is, however, an important step in the expression of the selective nigral toxicity of MPTP [2]. This study shows that isoquinoline derivatives are moderate to poor substrates for the dopamine reuptake system and that they are unlikely to be readily accumulated into dopaminergic neurones.

Measurement of the ability of isoquinoline derivatives to inhibit the specific uptake of [^{3}H]dopamine was employed because radiolabelled compounds were not available. This indirect means of determining substrate affinity for the dopamine reuptake system has been extensively used to study the uptake of MPTP/MPP^+, β-carbolines, and their structural analogs [32, 33]. Temperature was used to determine the nonspecific uptake of [^{3}H]dopamine into striatal synaptosomes because this is temperature-dependent and the accumulation of [^{3}H]dopamine is negligible at 0–4°C [2, 34]. However, the use of temperature to determine nonspecific uptake of [^{3}H]dopamine would also inhibit other active processes by which it might accumulate in synaptosomes. So, it is possible that a small proportion of the uptake defined as specific in this study represents other uptake mechanisms. The similarity between the proportion of total uptake inhibited by temperature, in this study, and by inclusion of uptake blockers such as mazindol or nomifensine, make this less likely [32–37]. It is also feasible that the ability of the isoquinoline derivative examined to inhibit [^{3}H]dopamine uptake does not reflect their active accumulation within synaptosomes by the dopamine reuptake system. The compounds might act extraneuronally to inhibit uptake of [^{3}H]dopamine in a manner similar to uptake site blockers, such as mazindol or nomifensine. At least for MPP^+ analogs, this seems unlikely because inhibition of [^{3}H]dopamine uptake correlates closely with their neurotoxic action [38].

The kinetics of [^{3}H]dopamine uptake into striatal synaptosomes was in agreement with previous reports [2]. MPP^+ inhibited the uptake of [^{3}H]dopamine in a concentration-dependent manner with an IC_{50} of 0.33 μM, in good agreement with values of 0.28 μM [34] and 0.40 μM [2] reported elsewhere. The isoquinoline derivatives also concentration-dependently inhibited the uptake of [^{3}H]dopamine, but less potently than MPP^+. Indeed, of the 15 isoquinoline derivatives studies, only the neutral compounds N-methyl-1,2,-3,4-tetrahydroisoquinoline, N-methyl-6-methoxy-1,2-dihydroisoquinoline, 7-methoxyisoquinoline, N-methylsalsolinol, and THIQ produced 50% inhibition of [^{3}H]dopamine uptake. Because, this is the first study on the substrate affinity of isoquinoline derivatives for the dopamine reuptake system, comparisons are difficult. However, in this study, the potency of salsolinol in inhibiting [^{3}H]dopamine uptake (39.9% at 100 μM) was in good agreement with previous reports that, at the same concentration, this compound inhibited [^{3}H]dopamine uptake by 28.4% [23].

QSAR or 3D-QSAR using CoMFA produced no clear correlation relating structure and activity of the isoquinoline derivatives. In contrast, qualitative SAR analysis revealed some interesting trends. The low activity of isoquinoline derivatives compared to MPP^+ was reminiscent of previous studies that failed to identify any MPTP/MPP^+ analog or β-carbolines with a greater affinity for the dopamine reuptake system than MPP^+ [32, 33, 38, 39]. The closest analog to MPP^+, N-Me-IQ^+, was devoid of activity. Indeed, Sayre and colleagues [20] reported that N-Me-IQ^+, at concentrations up to 300 μM, was unable to release [^{3}H]dopamine from preloaded rat striatal synaptosomes, compared to MPP^+ (30 μM), which caused 39% release [20]. The inactivity of N-Me-IQ^+ may be related to topographical reasons. Indeed, the intramolecular distance between the N- atom and the centroid of the benzene ring is close to 5.6 Å in the extended dopamine conformation and 6.0 Å in MPP^+, and it is only about 4.0 Å in N-Me-IQ^+. The other isoquinoline derivatives were all more active than N-Me-IQ^+, suggesting that they contain structural motifs that could, to some extent, compensate for the unfavourable N-centroid distance. These findings parallel the reports on the inhibition of synaptosomal [^{3}H]dopamine uptake by 29 β-carbolines, in which 2-methylnorharman, the β-carboline most similar to MPP^+, was found to be

some 50-fold less potent than MPP^+ and far from being the most potent compound of the group [32]. To rationalise the influence of structural motifs on the activity of isoquinoline derivatives, we took isoquinoline as the reference. By comparing pairs of compounds, favourble and unfavourable influences became apparent. This is possible because the influence of several functional groups is remarkably constant from one compound to the other. As far as the molecular skeleton is concerned, tetrahydroisoquinolines were consistently more active than dihydroisoquinolines and much more active than isoquinolines. This reflects reports that tetrahydropyridines were more potent inhibitors of [^{3}H]dopamine uptake into striatal synaptosomes than dihydropyridines [38]. Isoquinolinium cations (e.g. N-Me-IQ^+ and N-Me-7-OMe-IQ^+) were less active than neutral isoquinolines, suggesting that MPP^+ is possibly not the most active compound of its class. This is similar to findings that quaternisation of β-carbolines either reduced or had little effect on the degree of inhibition of [^{3}H]dopamine uptake [32]. Although an N-Me^+ group is unfavourable, it requires a lipophilic N-*n*-propyl substituent to compensate for the decrease in activity due to the positive charge. Indeed, substitution of a methyl for a *n*-propyl on 4-phenyl-1,2,3,6-tetrahydropyridines and 3-substituted β-carbolines increases potency for inhibition of [^{3}H]dopamine uptake [32, 33]. Methoxylation in positions 6- and 7- was found to have variable effects. A 7-methoxy group is favourable to activity and the 6-methoxy or 6,7-dihydroxy substituents decreased activity. These findings, again, reflect the influence of methoxylation on the activity of β-carbolines. Indeed, methoxylation of β-carbolines or 3,4-dihydro-β-carbolines in position 7- rather than in position 6- increases the ability of these compounds to inhibit [^{3}H]dopamine uptake into rat striatal synaptosomes [32].

Recently, we studied the effects of isoquinoline derivative on PC12 cells and compared their cytotoxicity with their potency in inhibiting complex I activity, glutamate + malate-supported respiration, and [^{3}H]dopamine uptake into rat striatal synaptosomes [40]. Cytotoxicity of these compounds directly correlate with their substrate affinity for the dopamine reuptake system (i.e. degree of inhibition of [^{3}H]dopamine uptake), but not inhibition of mitochondrial function [40]. This confirms previous reports by Johnson and colleagues who showed that the ability of various MPP^+ and β-carboline analogs to inhibit the uptake of [^{3}H]dopamine by rat striatal synaptosomes was directly correlated with their ability to cause irreversible dopamine depletion and nerve terminal damage in rat brain [38]. These findings suggest that the rate and extent to which isoquinoline derivatives are transported into dopaminergic neurones is an important and, perhaps, a rate-limiting factor in the expression of neurotoxicity.

In conclusion, the isoquinoline derivatives studies were moderate to poor substrates for the dopamine reuptake system and this may be a rate-limiting factor in their potential for toxicity to dopaminergic neurones. Thus, high concentrations of or prolonged exposure to isoquinoline derivatives may be necessary to cause neurodegeneration.

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