Biochemical Pharmacology, Vol. 56, pp. 921–933, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(98)00142-7

COMMENTARY

Isoquinoline Derivatives as Endogenous Neurotoxins in the Aetiology of Parkinson's Disease

Kevin St. P. McNaught, Pierre-Alain Carrupt,† Cosimo Altomare,‡ Saverio Cellamare,‡ Angelo Carotti,‡ Bernard Testa,† Peter Jenner*§ and C. David Marsden‖*

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

ABSTRACT. The cause of neurodegeneration in Parkinson's disease (PD) remains unknown. However, isoquinoline derivatives structurally related to the selective dopaminergic toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its active metabolite, 1-methyl-4-phenylpyridinim (MPP^+), have emerged as candidate endogenous neurotoxins causing nigral cell death in Parkinson's disease. Isoquinoline derivatives are widely distributed in the environment, being present in many plants and foodstuffs, and readily cross the blood–brain barrier. These compounds occur naturally in human brain where they are synthesized by non-enzymatic condensation of biogenic amines (e.g. catecholamines and phenylethylamine) with aldehydes, and are metabolized by cytochrome P450s and *N*-methyltransferases. In addition, isoquinoline derivatives are oxidized by monoamine oxidases to produce isoquinolinium cations with the concomitant generation of reactive oxygen species. Neutral and quaternary isoquinoline derivatives accumulate in dopaminergic nerve terminals via the dopamine re-uptake system, for which they have moderate to poor affinity as substrates. Several isoquinoline derivatives are selective and more potent inhibitors of NADH ubiquinone reductase (complex I) and α-ketoglutarate dehydrogenase activity in mitochondrial fragments than MPP^+, and lipophilicity appears to be important for complex I inhibition by isoquinoline derivatives. However, compared with MPP^+, isoquinoline derivatives are selective but less potent inhibitors of NADH-linked respiration in intact mitochondria, and this appears to be a consequence of their rate-limiting ability to cross mitochondrial membranes. Although both active and passive processes are involved in the accumulation of isoquinoline derivatives in mitochondria, inhibition of respiration is determined by steric rather than electrostatic properties. Compared with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or MPP^+, isoquinoline derivatives show selective but relatively weak toxicity to dopamine-containing cells in culture and following systemic or intracerebral administration to experimental animals, which appears to be a consequence of poor sequestration of isoquinoline derivatives by mitochondria and by dopamine-containing neurones. In conclusion, the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-like cytotoxic characteristics of isoquinoline derivatives and the endogenous/environmental presence of these compounds make it conceivable that high concentrations of and/or prolonged exposure to isoquinoline derivatives might cause neurodegeneration and Parkinson's disease in humans. BIOCHEM PHARMACOL **56**;8: 921–933, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. isoquinoline derivatives; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); 1-methyl-4-phenylpyridinium (MPP^+); 1,2,3,4-tetrahydroisoquinoline; neurotoxin; Parkinson's disease

PD¶ is an age-related neurodegenerative disorder characterized clinically by a slowly progressive disorder of movement, the cardinal symptoms of which are tremor, rigidity, and bradykinesia. The primary pathology of the disease is degeneration of the pigmented dopamine-containing neurones of the substantia nigra pars compacta in the midbrain, resulting in destruction of the nigrostriatal pathway and loss of caudate-putamen dopamine content, and the appearance of Lewy bodies in the surviving neurones [1, 2]. However, other neuronal systems (e.g. cerebral cholinergic, raphe serotoninergic, and locus coeruleus noradrenergic) are also destroyed to varying degrees, and this may be responsible

§ Corresponding author: Professor Peter Jenner, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Road, London, SW3 6LX, U.K. Tel. 44-171-333-4716; FAX 44-171-376-4736; E-mail: p.jenner@kcl.ac.uk

¶ *Abbreviations:* L-DOPA, L-3,4-dihydroxyphenylalanine; γ-GTP, γ-glutamyl-transpeptidase; α-KGDH, α-ketoglutarate dehydrogenase; MAO, monoamine oxidase; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; and TPB^-, tetraphenylboron.

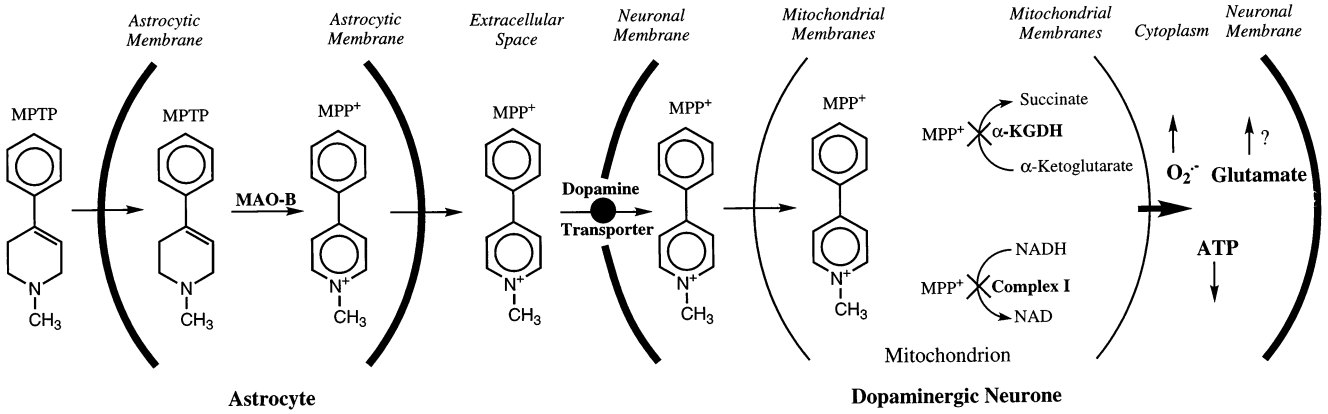

FIG. 1. Illustration of the mechanism of action of MPTP. In brain, MPTP is metabolized in astrocytes/serotoninergic neurones by MAO-B to produce its active metabolite MPP^+. MPP^+ is released into the extracellular space, and then accumulates in dopamine-containing neurones via the dopamine re-uptake system. In neurones, MPP^+ utilizes an energy-dependent mechanism for concentration within mitochondria where it inhibits complex I of the respiratory chain and α-KGDH of the tricarboxylic acid (TCA) cycle. Consequently, ATP depletion, elevation of reactive oxygen species, and possibly an increase in glutamate levels precede neuronal death.

for the occurrence of dementia and depression in advanced PD [1, 2]. The cause of nigral cell death in PD has been extensively sought, but remains unknown. Current concepts, however, suggest a genetic predisposition to the actions of an environmental or endogenous toxic substance, leading to cell death by a degenerative process involving oxidative stress and mitochondrial dysfunction [3]. The genetic contribution to PD is unclear, but this may be related to an inherited defect of xenobiotic metabolism leading to increased susceptibility to neurotoxic action [4]. This relates to findings that the activity of cytochrome P450 isoenzymes and *N*-acetyltransferase 2, and the metabolism of sulphur-containing compounds, may be impaired in PD [3–5]. Involvement of oxidative stress in the substantia nigra in PD is suggested by post-mortem evidence for free radical generation and reduced antioxidant defences. Thus, in the substantia nigra in PD, there are increased levels of iron with decreased or unaltered ferritin content, increased mitochondrial superoxide dismutase activity, decreased levels of GSH, and increased γ-GTP activity [6–9]. In addition, there are decreased levels of polyunsaturated fatty acids with elevated levels of malondialdehyde and lipid hydroperoxides, and increased levels of 8-hydroxy-2-deoxyguanosine, indicative of elevated lipid peroxidation and DNA fragmentation, respectively [10, 11]. The contribution of mitochondrial dysfunction to the pathogenesis of nigral cell death in PD is evident from inhibition of NADH ubiquinone reductase (complex I) activity and reduced immunostaining for the tricarboxylic acid (TCA) cycle enzyme α-KGDH [12, 13]. There is, at present, no convincing evidence to suggest that oxidative stress or mitochondrial dysfunction is related to abnormal genetic function. Rather, these biochemical changes appear to result from a neurotoxin acting in genetically vulnerable individuals.

No toxic substance thought to be responsible for neurodegeneration in PD has been identified, despite evidence that the occurrence of the disease parallels industrialization, the use of agrochemicals, rural residence, and the drinking of well water [14]. Indeed, several chemical agents can induce an irreversible (e.g. manganese, carbon monoxide, and carbon dioxide) or a reversible (e.g. phenothiazines and reserpine) parkinsonian-like syndrome in humans, but the clinical and pathological characteristics of these disorders are different from those of idiopathic PD [15, 16]. However, the discovery that MPTP induces a parkinsonian syndrome in humans and experimental animals by selective destruction of the nigrostriatal pathway supports the role of neurotoxic action in the cause of PD [17, 18]. In the brain, MPTP is metabolized by glial MAO-B to produce its active metabolite MPP^+ (Fig. 1) [19]. MPP^+ is actively accumulated into dopaminergic neurones via the dopamine re-uptake system prior to its energy-dependent concentration within mitochondria (Fig. 1) [20, 21]. MPP^+ induces cell death by selective inhibition of complex I and α-KGDH activity, resulting in ATP depletion and the concomitant generation of reactive oxygen species and possibly glutamate (Fig. 1) [22–24]. However, MPTP or closely related analogs could not be the cause of PD because, for example, antibodies to MPTP/MPP^+ show no immunoreactivity in brain in PD [25]. Nevertheless, the discovery of MPTP suggests that similar toxins may accumulate in the brain and cause PD, and this has stimulated the search for MPTP-like agents. Isoquinoline derivatives were discovered as possible MPTP-like endogenous/environmental neurotoxins causing PD [26, 27]. The initial interest in these compounds arose from their structural similarity to MPTP/MPP^+. But ongoing efforts to describe their neurotoxicity indicate that although isoquinoline derivatives possess many of the cytotoxic characteristics of MPTP, they also have unique properties relevant to the aetiology of PD [26]. This review examines the chemistry, distribution, and neurotoxicity of isoquinoline derivatives, and their possible involvement in the aetiology of PD.

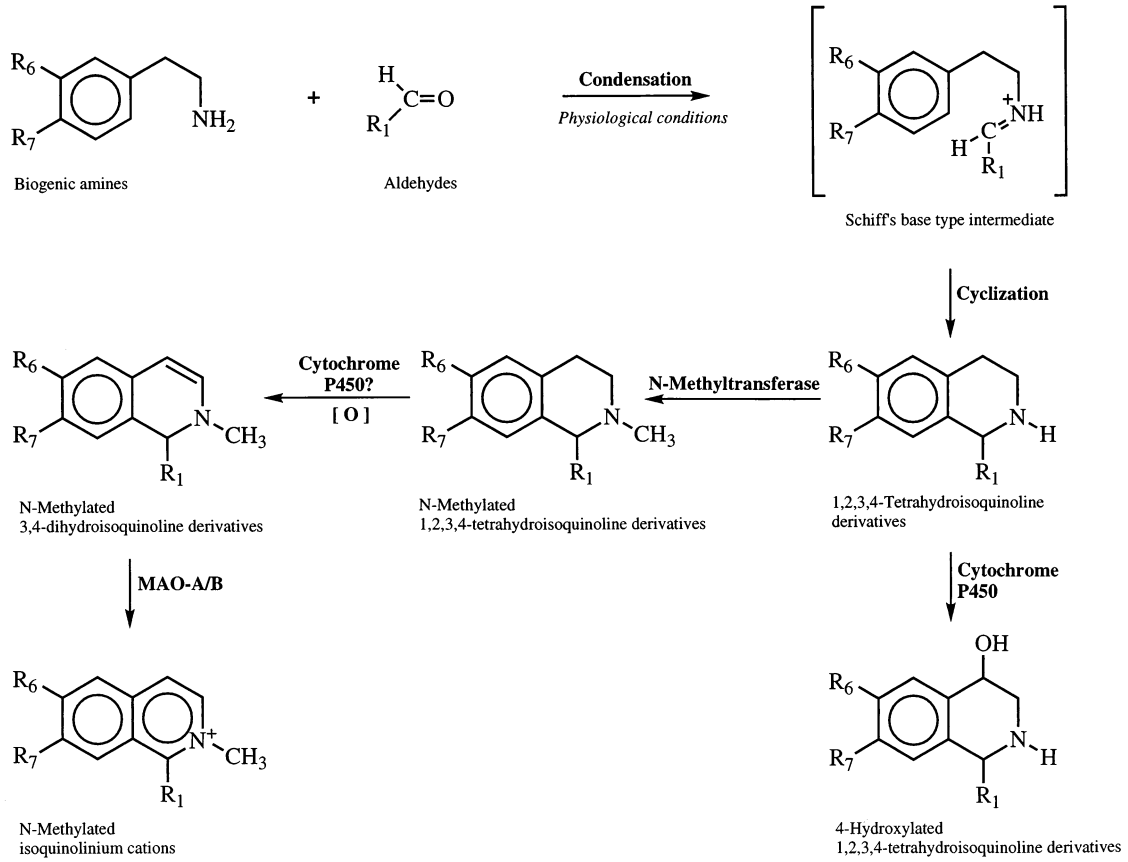

FIG. 2. Pictet–Spengler biosynthesis and enzyme-mediated metabolism of isoquinoline derivatives. One example of such a reaction, under physiological conditions in the human brain, would be dopamine condensing with acetaldehyde non-enzymatically to produce racemic (*R*- and *S*-enantiomers) 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol). 1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline is converted by *N*-methyltransferase to produce 1,2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, which, in turn, is oxidized (possibly by cytochrome P450) to produce 1,2-dimethyl-3,4-dihydroisoquinoline. 1,2-Dimethyl-3,4-dihydroisoquinoline is metabolized by MAO-A/B to produce the 1,2-dimethyl-6,7-dihydroxyisoquinolinium cation. Debrisoquine (2-amidino-1,2,3,4-tetrahydroisoquinoline) is hydroxylated in hepatic microsomes and possibly in brain by the cytochrome P450 isoenzyme CYP2D6 to produce 4-hydroxydebrisoquine (2-amidino-4-hydroxy-1,2,3,4-tetrahydroisoquinoline).

CHEMISTRY AND OCCURRENCE OF ISOQUINOLINE DERIVATIVES

Basic Chemistry

Isoquinoline derivatives refer to isoquinoline itself, substituted congeners (e.g. 6-methoxyisoquinoline) and the various reduced species (1,2-/3,4-dihydroisoquinolines and 1,2,3,4-tetrahydroisoquinolines), all of which may occur in their neutral or charged quaternary (isoquinolinium ion) form (Figs. 2 and 3). These compounds are heterocycles in which a benzene ring and a pyridine ring are fused through carbon, and are formed from Pictet–Spengler non-enzymatic condensation of catecholamines (e.g. dopamine and L-DOPA) with aldehydes (Figs. 2 and 3) [28, 29].

Environmental Distribution

The history of isoquinoline derivatives emerged following the discovery of isoquinoline (and various methylated derivatives) in 1885 as a constituent of coal tar [30, 31]. In the early 1900s, salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) was isolated from the desert plant *Salsola richteri*, of the family *Chenopodiaceae*, present in the Turkmenistan desert [30, 31]. This compound has also been detected in the pulp (40 μg/g) and the peel (260 μg/g) of bananas. 1,2,3,4-Tetrahydroisoquinolines have been found in *Cactus* species and several species of *Guatteria* (Annonaceae) present in the Amazon forest [30, 31]. More recently, isoquinoline derivatives have been reported to occur in *Guatteria ouregtou*, *Magnolia salicifolia*, *Eschscholtzia tenuifolia*, *Papaver orientale*, and *P. somniferum* [30, 31]. There is some evidence to suggest that isoquinoline derivatives serve as precursors in the natural biosynthesis of opiates in the poppy. Indeed, papaverine, morphine, and emetine are tetrahydroisoquinoline alkaloids, and are found in poppies [30, 31].

Occurrence in Foodstuffs

Isoquinoline derivatives occur in various foodstuffs. 1,2,3,4-Tetrahydroisoquinoline is present in flour (0.52 ng/g), banana (2.2), cheese (5.2), broiled sardine (0.96), broiled beef (1.3), and the yolk (1.8) and white (2.2) of boiled eggs

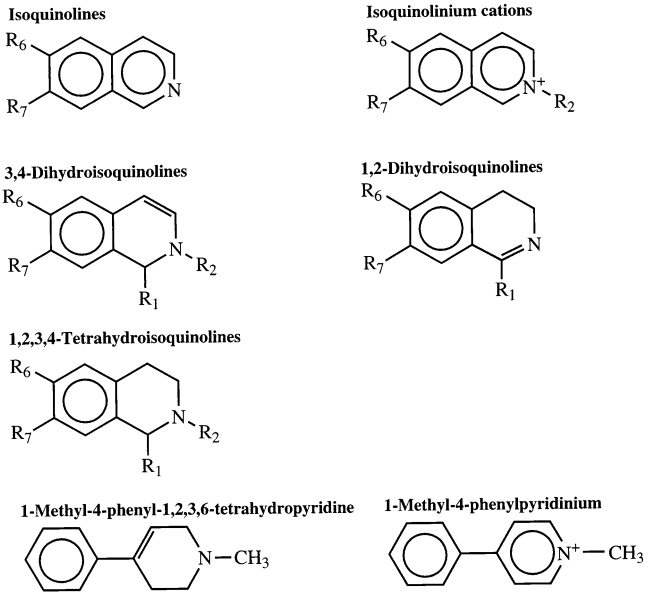

FIG. 3. Structures of isoquinoline derivatives, MPTP and MPP$^+$. The effects of a range of compounds from these classes of isoquinoline derivatives on mitochondrial function, affinity for the dopamine re-uptake system, and toxicity to dopamine-containing cells are presented in Table 1.

[32, 33]. 1-Methyl-1,2,3,4-tetrahydroisoquinoline (6.5 ng/g) and 1,2,3,4-tetrahydroisoquinoline (0.8 ng/g) occur in cocoa [32, 33]. 1,2,3,4-Tetrahydroisoquinolines are also present in various alcoholic beverages including wine (0.56 ng/g), beer (0.36), and whisky (0.73), and in milk (3.3) [32, 33].

Occurrence In Vivo

Isoquinoline derivatives were first shown to occur in humans around 1970. The compounds are normally synthesized under physiological conditions and have been detected in mammalian adrenal glands, kidneys, liver, brain, urine, blood, and cerebrospinal fluid (CSF) [28, 29]. Indeed, 1,2,3,4-tetrahydroisoquinoline (5–7 ng/g), *N*-methyl-1,2,3,4-tetrahydroisoquinoline (1–3 ng/g), salsolinol, and other isoquinoline derivatives are found in the brain of various animal species and in humans [34–46]. These compounds are synthesized *in vivo* by Pictet–Spengler non-enzymatic condensation of biogenic amines (e.g. catecholamines and phenylethylamines) with aldehydes (or with α-keto acids followed by decarboxylation). Recent studies suggest that some isoquinoline derivatives may also be formed enzymatically in mammalian brain [26]. Isoquinoline derivatives are metabolized by various enzymes including *N*-methyltransferase and MAO (but very slowly compared with MPTP) to produce *N*-methylated derivatives and isoquinolinium cations, respectively (Fig. 2) [38–41]. Isoquinoline derivatives are also metabolized in the brain and liver by cytochrome P450 isoenzymes to produce 4-hydroxylated products (Fig. 2) [47]. Recent reports suggest that isoquinoline derivatives occur intraneuronally as well as extraneuronally, and that their synthesis, metabolism, and levels are selectively elevated in dopaminergic neurones of the substantia nigra compared with other brain regions [38, 39]. Indeed, the (*R*)-enantiomer of salsolinol in human brain is methylated to *N*-methylsalsolinol, which, in turn, is oxidized to 1,2-dimethyl-6,7-dihydroxyisoquinolinium cation, that, unlike salsolinol and its methylated derivative, could only be detected in the substantia nigra (Fig. 2) [38, 39]. Some isoquinoline derivatives are able to cross the blood–brain barrier, and this may represent additional means of CNS accumulation [42, 43].

NEUROTOXICOLOGY OF ISOQUINOLINE DERIVATIVES

Biological Functions

The physiological function of isoquinoline derivatives is poorly understood. However, there is evidence to suggest that these compounds function as neurotransmitters/neuromodulators (e.g. dopamine receptor antagonists, and β-adrenergic receptor agonists and antagonists), and may be involved in the regulation of monoamine function through reversible and competitive inhibition of enzymes involved in monoamine synthesis and metabolism (e.g. tyrosine hydroxylase, L-aromatic amino acid decarboxylase, catechol-*O*-methyltransferase, and MAO-A and -B) [28, 29, 48, 49]. In human disease, isoquinoline derivatives have been implicated in the pathophysiology of alcohol addiction, monoamine-related neurological and psychiatric disorders (e.g. schizophrenia and depression), and in phenylketonuria, but there is little or no convincing evidence to support such roles [28, 29, 31, 48].

Effects on Mitochondrial Enzymes

Impairment of complex I activity and α-KGDH in PD and by MPP$^+$ prompted the examination of the effects of isoquinoline derivatives on mitochondrial function. Suzuki and colleagues [50, 51] first reported that 1,2,3,4-tetrahydroisoquinoline and its oxidized quaternary cation, *N*-methylisoquinolinium, concentration-dependently inhibited the activity of complex I but had no effect on complexes II, III, or IV in mouse brain mitochondrial fragments. Subsequently, we conducted a comprehensive study to determine the selectivity, potency, and molecular requirements of a range of structurally closely related isoquinoline derivatives (eleven isoquinolines, two dihydroisoquinolines, and nine 1,2,3,4-tetrahydroisoquinolines) on respiratory and non-respiratory chain enzymes in mitochondrial fragments (Fig. 3) [52, 53]. Most of the compounds studied selectively inhibited complex I and α-KGDH activity in a concentration-dependent manner, and several isoquinoline derivatives were markedly more potent than MPP$^+$ (Table 1). Although no clear structure–activity relationship was found for the inhibition of complex I or α-KGDH activity, lipophilicity, as with MPP$^+$

TABLE 1. Effects of isoquinoline derivatives on mitochondrial function, affinity for the dopamine re-uptake system, and toxicity to dopamine-containing cells

Compounds*	Inhibition of α-KGDH activity† (IC_{50}, mM)	Inhibition of complex I activity‡ (IC_{50}, mM)	Inhibition of mitochondrial respiration§ (%, Mean)	Inhibition of [^{3}H]dopamine uptake‖ (%, Mean)	Toxicity to PC12 cells¶ (EC_{50}, μM)	Nigral cell death *in vivo*** (%, Mean)
Isoquinolines						
Isoquinoline	6.5	0.75	31.3	36.0	640	—
6-Methoxyisoquinoline	—	0.5	100	—	—	—
7-Methoxyisoquinoline	—	1.1	48.5	50.0 (10.0)	—	—
6,7-Dimethoxyisoquinoline	—	15	0	—	—	—
6,7-Methylenedioxyisoquinoline	—	0.39	51.4	37.0	600	—
Isoquinolinium cations						
N-Methylisoquinolinium	—	1.3	71.4	3.3	1050	—
N-Propylisoquinolinium	3.0	—	78.7	32.8	—	0
N-Methyl-6-methoxyisoquinolinium	—	0.7	100	52.4 (10)	—	—
N-Methyl-7-methoxyisoquinolinium	—	0.85	74.7	7.0	—	—
N-Methyl-6,7-dimethoxyisoquinolinium	—	2.3	13.4	—	—	41.5
N-Methyl-6,7-methylenedioxyisoquinolinium	—	15.4	100	7.0	—	—
Dihydroisoquinolines						
6,7-Dimethoxy-1-styryl-3,4-dihydroisoquinoline	—	—	—	6.6	—	0
N-Methyl-6-methoxy-1,2-dihydroisoquinoline	—	—	—	—	—	—
1,2,3,4-Tetrahydroisoquinolines						
1,2,3,4-Tetrahydroisoquinoline	18.2	22	43.2	50.0 (50.0)	820	20.2
N-Methyl-1,2,3,4-tetrahydroisoquinoline	2.0	4.3	17.6	61.1 (8.0)	—	—
6-Methoxy-1,2,3,4-tetrahydroisoquinoline	—	0.38	—	—	—	—
N-Methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline	—	0.36	9.5	—	—	—
7-Methoxy-1,2,3,4-tetrahydroisoquinoline	—	1.1	—	—	—	—
6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline	—	Not attained	0	23.5	—	—
N-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline	Not attained	8.9	12.5	39.3	—	—
1,2-Dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline	—	4.6	13	72.7 (37.0)	—	—
1-Methyl-4-phenylpyridinium (MPP^+)	18.9	4.1	100	100 (0.33)	375	88.3

*See Fig. 3 for structures.

†Inhibition of α-KGDH activity in rat brain mitochondrial fragments by isoquinoline derivatives and MPP^+. Results are presented as mean IC_{50} (mM), and were obtained from Ref. 52.

‡Inhibition of complex I activity in rat brain mitochondrial fragments by isoquinoline derivatives and MPP^+. Results are presented as mean IC_{50} (mM), and were obtained from Ref. 53.

§Inhibition of glutamate + malate supported state 3 respiration in intact isolated rat liver mitochondria by isoquinoline derivatives and MPP^+ at a concentration of 0.5 mM and an incubation time of 20 min. Results are presented as mean percent inhibition, and were obtained from Ref. 63.

‖Inhibition of [^{3}H]dopamine uptake into rat striatal synaptosomes by isoquinoline derivatives and MPP^+ at a concentration of 100 μM. Results are presented as mean percent inhibition (figures in parentheses represent IC_{50}, μM), and were obtained from Ref. 72.

¶Cytotoxicity of isoquinoline derivatives and MPP^+ to cultured PC12 cells. Results are presented as mean EC_{50} (μM) for the release of lactate dehydrogenase activity after 3 days of treatment, and were obtained from Ref. 77.

**Cytotoxicity of isoquinoline derivatives (150 nmol/24 hr) and MPP^+ (33 nmol/24 hr) to tyrosine hydroxylase-positive neurones following continuous (7 days) unilateral supranigral infusion in conscious adult rats. Results are presented as mean reduction in the number of ipsilateral TH-positive neurones in the substantia nigra pars compacta, and were obtained from Ref. 84.

analogs, appeared to be important for inhibition of complex I activity. Indeed, recently, the presence of dimethoxy residues in the catechol ring of papaverine has been suggested to be responsible for the increased potency of papaverine in inhibiting rat brain complex I activity compared with tetrahydropapaveroline [54]. Surprisingly, however, the kinetics of inhibition of complex I and α-KGDH activity by isoquinoline derivatives have not been exam-

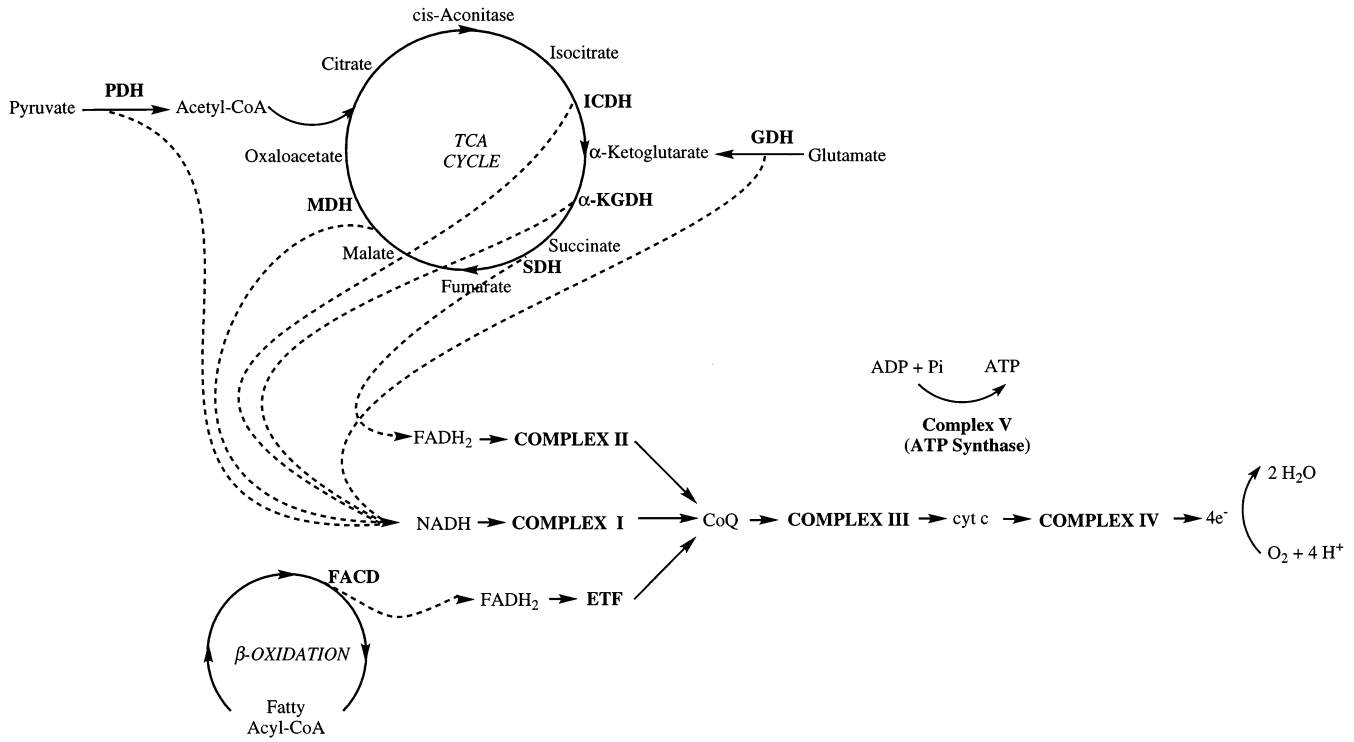

FIG. 4. Functional organization of the mitochondrial respiratory chain and the provision of NADH and $FADH_2$ as substrates. Abbreviations: GDH, glutamate dehydrogenase; ICDH, isocitrate dehydrogenase; α-KGDH, α-ketoglutarate dehydrogenase; MDH, malate dehydrogenase; SDH, succinate dehydrogenase; CoQ, coenzyme Q (ubiquinone); cyt *c*, cytochrome *c*; FACD, fatty acyl-CoA dehydrogenase; ETF, electron-transferring flavoprotein; complex I, NADH ubiquinone reductase; complex II, succinate cytochrome *c* reductase; complex III, ferrocytochrome *c* reductase; and complex IV, cytochrome *c* oxidase. Note that only enzymes that generate NADH and $FADH_2$ in the TCA cycle and the β-oxidation pathway are shown.

ined. Determination of this is important because the marked mitochondrial inhibitory potency of rotenone (the most potent inhibitor of complex I known) is attributed to its slow dissociation from its binding site on complex I [55]. MPP^+ binds partially reversibly and competitively to complex I, and, therefore, it is a less effective inhibitor of mitochondrial function. So, it is necessary to determine the kinetic parameters for inhibition of complex I and α-KGDH activity by isoquinoline derivatives to fully assess their mitochondrial toxicity.

The ability of isoquinoline derivatives to inhibit α-KGDH activity is important for their mitochondrial toxicity and involvement in PD. Complex I is one of a few enzymatic pathways by which reducing equivalents enter the respiratory chain (Fig. 4). Therefore, it is unlikely that specific inhibition of complex I activity can produce mitochondrial failure. When complex I is defective, complex II serves as an alternative electron transfer pathway, but it is not inhibited in PD [12]. However, because α-KGDH catalyses the oxidation of α-ketoglutarate to succinate, which serves as a substrate for complex II, inhibition of α-KGDH would inhibit electron transfer via complex II. Furthermore, electron transfer via complex I would be partially inhibited since the metabolism of α-ketoglutarate by α-KGDH also produces NADH, which enters the respiratory chain via complex I (Fig. 4). Overall, the general function of the TCA cycle would be impaired because α-KGDH is a rate-limiting component. Thus, combined inhibition of α-KGDH and complex I by isoquinoline derivatives appears more likely to cause mitochondrial failure and neurodegeneration in PD. It is not yet known if isoquinoline derivatives can inhibit complex V (ATP synthase) or other crucial enzymes involved in mitochondrial energy metabolism, such as isocitrate dehydrogenase, malate dehydrogenase, and pyruvate dehydrogenase, although none of these appear to be inhibited by MPP^+ (Fig. 4) [56].

Mitochondrial Accumulation

Complex I and α-KGDH in mitochondrial fragments can be readily inhibited by isoquinoline derivatives, MPP^+, and related analogs because the enzymes and their inhibitor binding sites are easily accessible [57]. Thus, inhibition of complex I or α-KGDH activity in mitochondrial fragments does not indicate whether or not isoquinoline derivatives can inhibit mitochondrial function in intact mitochondria or whether these compounds are concentrated by mitochondria in a manner similar to MPP^+. Determination of this is important since 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

[58–60]. Indeed, some MPTP/MPP^+ analogs, such as 4-phenylpyridines, which are potent inhibitors of complex I activity in mitochondrial fragments, are without effect on intact mitochondria since they do not possess the necessary steric/electrostatic properties for mitochondrial accumulation [57]. Conversely, some MPTP/MPP^+ analogs that are weak inhibitors of complex I activity in mitochondrial fragments, such as *N*-methylpyridinium cations including MPP^+, become potent inhibitors of respiration due to their active mitochondrial concentration [57]. Hence, the effects of isoquinoline derivatives on mitochondrial respiration have been studied. 1,2,3,4-Tetrahydroisoquinoline and *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 (but not succinate) and reduce ATP synthesis in intact mouse brain mitochondria [61, 62]. Recently, we determined the selectivity, potency, and structural requirements of isoquinoline derivatives for inhibition of respiration in intact mitochondria by studying a range of neutral and quaternary isoquinoline derivatives (Fig. 3) [63]. Most of the compounds examined inhibited glutamate + malate, but not succinate + rotenone or tetramethylparaphenylenediamine (TMPD) + ascorbate supported respiration (Table 1). These findings support previous studies showing that isoquinoline derivatives are selective inhibitors of complex I and α-KGDH activity in mitochondrial fragments (Fig. 4). In our study, none of the isoquinoline derivatives were found to be as potent as MPP^+ in inhibiting respiration. However, recently papaverine was shown to be more potent than MPP^+ in inhibiting mitochondrial respiration [54]. Isoquinoline derivatives are less potent in inhibiting respiration in intact mitochondria than in impairing complex I activity in mitochondrial fragments, suggesting that they are not accumulated by mitochondria as avidly as MPP^+ (Table 1) [53, 63]. Recently, isoquinoline and 1,2,3,4-tetrahydroisoquinoline were shown to inhibit ATP synthesis supported by pyruvate + malate or α-ketoglutarate + malate, but not succinate + rotenone, in PC12 cells and SK-N-MC dopaminergic cell lines [64].

Studies using intact mitochondria have provided some clear insights into the structural requirements for inhibition of mitochondrial function [63]. Mitochondrial respiration is inhibited by both charged and neutral isoquinoline derivatives, suggesting that isoquinoline derivatives utilize both active and passive processes to enter mitochondria, although the quaternary nitrogen moiety of the isoquinolinium cations favours mitochondrial accumulation and inhibition of respiration [63]. The inhibitory potency of isoquinoline derivatives towards mitochondrial respiration is determined more by their steric than their electrostatic properties [63]. Furthermore, a hypothetical binding site for isoquinoline derivatives has been deduced, which may be related to the rate-limiting transport process rather than to enzyme inhibition [63]. The mechanism of inhibition of mitochondrial respiration and complex I activity by isoquinoline derivatives has not been investigated. One possibility is to determine the effects of TPB^-, because this compound acts by ion pairing with MPP^+ to facilitate penetration into mitochondria as well as aiding access to the hydrophobic inhibitory site on complex I [58, 59]. Examining the effects of TPB^- on the inhibition of mitochondrial function by isoquinoline derivatives may provide further insights into the mechanism of their mitochondrial accumulation and the nature of the enzyme binding site(s) and mechanism(s) of inhibition.

Generation of Free Radicals

Isoquinoline derivatives may cause oxidative stress, but only a few studies have addressed this issue. The non-enzymatic auto-oxidation of *N*-methylsalsolinol produces isoquinolinium cations and hydroxyl radicals, a process that is inhibited by the antioxidants ascorbate and glutathione, but is enhanced by Fe^{2+} [65, 66]. *N*-Methylsalsolinol induces DNA damage and apoptotic cell death in human dopaminergic neuroblastoma SH-SY5Y cells by a mechanism that involves the generation of hydroxyl radicals [67]. The antioxidants catalase and GSH protected these cells from *N*-methylsalsolinol-induced DNA fragmentation [67]. In addition, deprenyl and semicarbazide inhibited DNA damage in SH-SY5Y cells, and this was attributed to hydroxyl radical scavenging and the metabolism of *N*-methylsalsolinol by these compounds, respectively [67]. A recent study showed that isoquinoline and 1,2,3,4-tetrahydroisoquinoline induced apoptotic cell death in PC12 cells and SK-N-MC dopaminergic cell lines by a mechanism involving generation of free radicals, perhaps secondary to inhibition of the mitochondrial respiratory chain [64]. Apoptosis in these cells was prevented by the antioxidants, dihydrolipoic acid, *N*-acetylcysteine, and pyrrolidine dithiocarbamate [64]. Isoquinoline derivatives may also impair antioxidant defence mechanisms. Papaverine was once used as a smooth muscle relaxant to treat various diseases, but its use is now limited due to the tendency to cause hepatotoxicity, which may be attributed to its ability to alter glutathione levels [68]. The possibility of release of free radicals from the mitochondrial respiratory chain following inhibition by isoquinoline derivatives remains to be explored. Determination of this is important since the inhibition of complex I activity by MPP^+ is accompanied by the release of reactive oxygen species from the respiratory chain [24]. These, in turn, produce partial irreversible inactivation of complex I, which is promoted and prevented by free radical generators and scavengers, respectively [24]. Therefore, free radical production and impaired antioxidant defences may contribute to the mechanism of action of isoquinoline derivatives in causing neurodegeneration.

Accumulation in Dopaminergic Neurones

Uptake of MPP^+ via the dopamine re-uptake system is required for dopaminergic toxicity (Fig. 1). Several studies have suggested that isoquinoline derivatives may accumulate in dopaminergic neurones in a manner similar to MPP^+. Tritiated 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and 1-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline accumulate in rat brain synaptosomes in a concentration-dependent manner [69]. 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 [69]. Similarly, *N*-methylisoquinolinium has been shown to accumulate in rat striatal slices and 2-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline depletes striatal dopamine in rat brain [70, 71]. Recently, 1-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline and 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline were shown to accumulate in isolated rat striatal synaptosomes via the dopamine re-uptake system [44]. Uptake was concentration-dependent and was attenuated by inhibitors of the dopamine re-uptake system [44]. The substrate affinity of isoquinoline derivatives for the dopamine re-uptake system, assessed by determining their effects on the uptake of [^{3}H]dopamine into rat striatal synaptosomes, showed that isoquinoline derivatives were moderate to poor substrates for the dopamine re-uptake system (Table 1) [72]. This suggests that low affinity of isoquinoline derivatives for the dopamine re-uptake system may be a rate-limiting factor in nigral toxicity.

Previous studies have shown that the selective dopaminergic toxicity of MPTP via MPP^+ is due, in part, to its relatively high affinity for the dopamine re-uptake system and poor affinity for other neurotransmitter uptake systems, including those for acetylcholine, serotonin, and noradrenaline [18, 20]. Therefore, it is of particular importance to determine the affinity of isoquinoline derivatives for these and other neurotransmitter uptake systems.

Toxicity to Cells in Culture

Using cells in culture, *N*-methylisoquinolinium, 1,2,3,4-tetrahydroisoquinoline, papaverine, and tetrahydropapaverine exerted dose-dependent toxicity towards tyrosine hydroxylase, but not γ-aminobutyric acid-like, immunoreactive cells in rat embryonic mesencephalic culture and ventral mesencephalic striatal co-culture [73–75]. 1,2-Dimethyl-6,7-dihydroxyisoquinolinium, but not its parent compound, *N*-methylsalsolinol, causes necrotic cell death in human dopaminergic neuroblastoma SH-SY5Y cells [67]. The cytotoxicity of a range of isoquinoline derivatives to human dopaminergic neuroblastoma SH-SY5Y cells showed that catechol isoquinoline derivatives (e.g. salsolinol and 1,2-dimethyl-6,7-dihydroxyisoquinolinium) were more potent than their non-catechol congeners (1,2,3,4-tetrahydroisoquinoline and 1,2-dimethylisoquinolinium) in conferring toxicity to SH-SY5Y cells [76]. A recent study showed that isoquinoline and 1,2,3,4-tetrahydroisoquinoline caused apoptotic cell death in PC12 cells and SK-N-MC dopaminergic cell lines by a mechanism that appears to involve inhibition of the mitochondrial respiratory chain and generation of free radicals [64]. In our studies, the toxicity of the isoquinoline derivatives, isoquinoline, *N*-methylisoquinolinium, 6,7-methylenedioxyisoquinoline, and 1,2,3,4-tetrahydroisoquinoline to PC12 cells, directly correlated with their affinity for the dopamine re-uptake system (but not mitochondrial inhibitory potency) which appeared to be the limiting factor in their relatively weak toxicity (Table 1) [77].

In Vivo *Neurotoxicity*

Studies of the *in vivo* neurotoxicity of isoquinoline derivatives have produced conflicting results. Subcutaneous administration of 1,2,3,4-tetrahydroisoquinoline to marmosets (50 mg/kg daily for 11 days) or squirrel monkeys (20 mg/kg for 104 days) was reported to produce a parkinsonian syndrome with biochemical (inhibition of nigral tyrosine hydroxylase activity), neurochemical (reduction in nigral dopamine levels), and behavioural changes indicative of neuronal toxicity [27, 78]. Also, chronic administration of 1-benzyl-1,2,3,4-tetrahydroisoquinoline was reported to induce parkinsonism in primates [46]. In contrast, subcutaneous administration of *N*-methyl-1,2,3,4-tetrahydroisoquinoline (10 mg/kg twice daily for 100 days) to aged rhesus or crab-eating monkeys produced no pathological abnormalities in the substantia nigra [79]. Similarly, chronic infusion (30 mg over 4 weeks, then 150 mg over the next 4 weeks) of *N*-methylisoquinolinium and *N*-methylnorsalsolinol into the lateral ventricles of macaque monkeys again failed to produce pathological change in the substantia nigra [79]. In black C57 mice, subcutaneous injection of 1,2,3,4-tetrahydroisoquinoline (50 mg/kg daily for 70 days) was not toxic to nigral dopamine-containing neurones even though these cells are susceptible to MPP^+ [80]. Indeed, treatment of black C57 mice for 26 days with the maximum tolerated dose of 1,2,3,4-tetrahydroisoquinoline (cumulative dose, 2120 mg/kg, s.c.) produced no effect on striatal dopamine content [81]. Furthermore, treatment of black C57 mice for 5 days with the maximum tolerated dose of 1-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline or 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline produced some or no behavioural abnormality indicative of parkinsonism [44]. Indeed, unilateral injection (8 μg) of *N*-methylnorsalsolinol into the medial forebrain bundle of rats did not produce cell death [82].

These discrepancies may relate to the route, dose, and period of administration, and to the physiochemical properties (e.g. lipophilicity) of the particular isoquinoline derivatives studied. For example, while systemic administration of MPTP to primates produces a marked nigrostriatal toxicity due to its ability to cross the blood–brain barrier, isoquinoline derivatives, such as salsolinol [83], 1,2,3,4-tetrahydroisoquinoline and *N*-methyl-1,2,3,4-tetra-

hydroisoquinoline [42, 43], are less lipophilic and may not enter the brain to the same degree. However, isoquinoline derivatives are synthesized and metabolized in brain, and so their direct toxicity to nigral cells needs to be assessed [38–41]. Indeed, direct intracerebral (intranigral, intraventricular, or intrastriatal) application of MPP^+ is required to produce destruction of the substantia nigra pars compacta and nigrostriatal pathway. As such, we examined the effects of chronic (7 days; 150 nmol/day) unilateral supranigral infusion of *N*-*n*-propylisoquinolinium, 6,7-dimethoxy-1-styryl-3,4-dihydroisoquinoline, *N*-methyl-6,7-dimethoxyisoquinolinium and 1,2,3,4-tetrahydroisoquinoline compared with those of MPP^+ in rats (Fig. 3) [84]. The latter two isoquinoline derivatives produced destruction of the nigrostriatal system, although they were less toxic than MPP^+ (Table 1). In addition, while affinity for the dopamine re-uptake system appeared to be the limiting factor in the expression of toxicity, inhibition of mitochondrial function was the primary, if not the only mechanism by which these compounds exerted toxicity [84]. These findings suggest that high concentrations of and/or prolonged exposure to isoquinoline derivatives may be necessary to produce significant dopaminergic toxicity. This, however, does not discount the possible involvement of isoquinoline derivatives in the pathophysiology of PD since the slowly progressive nature of the disease, which often spans several decades, suggests that a mild but prolonged degenerative process may be responsible for neuronal loss in the disease.

CLINICAL RELEVANCE

Can Isoquinoline Derivatives Cause Neurodegeneration in PD?

The scarcity of data on the levels of isoquinoline derivatives in the brains of untreated PD patients makes it difficult to relate their potency in inhibiting mitochondrial function, affinity for the dopamine re-uptake system, and their toxicity to dopamine-containing cells in culture and in experimental animal models, to their potential for causing neurodegeneration in PD. However, even if only low concentrations of isoquinoline derivatives are found in PD and if they have only weak neurotoxic properties, the effects could be cumulative over a prolonged period of exposure. This is important considering that the presymptomatic and symptomatic stages of PD span many years. Alternatively, the concentrations of isoquinoline derivatives in brain could be elevated due to increased synthesis or impaired metabolism, and there is evidence to suggest that this could be the case in PD. For example, the levels of 1,2,3,4-tetrahydroisoquinoline, tetrahydropapaveroline, and salsolinol are elevated in the brain and urine of parkinsonian patients [36, 37, 85]. Although this may be related to L-DOPA treatment, resulting in increased synthesis of isoquinoline derivatives, impaired metabolism of these compounds cannot be excluded. Indeed, metabolism of debrisoquine (2-amidino-1,2,3,4-tetrahydroisoquinoline) and the activity of cytochrome P450 isoenzymes may be impaired in some parkinsonian patients, although this is controversial [4, 86].

Is There Evidence That Isoquinoline Derivatives Cause Neurodegeneration in PD?

If isoquinoline derivatives are responsible for neuronal death in PD, then it is reasonable to presume that increased risk of developing the disease and the occurrence of PD would be associated with exogenous/endogenous factors increasing brain concentrations of these compounds and/or imparting vulnerability to their toxic actions at normal or elevated levels. Since isoquinoline derivatives are widely distributed in the environment and are able to cross the blood–brain barrier, it is reasonable to suspect that the incidence and prevalence of PD could be higher in countries/communities where these sources of isoquinoline derivatives constitute a part of the normal diet. However, there is no evidence to suggest that the occurrence of PD is related to diet in any country [14]. Indeed, plants and fruits containing high levels of isoquinoline derivatives predominate in extreme climates like the desert and tropics, but the occurrence of PD in these regions shows little or no difference compared with other countries [31, 87]. In the brain of animals and humans, increased levels of isoquinoline derivatives occur following alcohol consumption (which is metabolized *in vivo* to produce aldehydes), elevation of dopamine levels in schizophrenics, or following L-DOPA administration [28, 48]. However, there is no evidence to suggest that chronic alcoholism leading to Wernicke-Korsakoff syndrome or schizophrenia is associated with an increased risk of developing PD. In parkinsonian patients undergoing chronic L-DOPA therapy, the levels of some isoquinoline derivatives are increased in the brain and urine, but this may be due to the L-DOPA therapy itself. It is therefore necessary to determine the levels of isoquinoline derivatives in untreated PD subjects compared with normal individuals to determine the correlation between the levels of isoquinoline derivatives and occurrence of the disease. Indeed, in recent years, there has been some concern that, despite L-DOPA being the most effective symptomatic therapeutic agent for treating PD, it may also hasten the underlying neurodegenerative process [88, 89]. This is thought to be due to increased synthesis and metabolism of dopamine-generating free radicals and other toxic species [88, 89]. However, increased production of isoquinoline derivatives cannot be excluded. Interestingly, a recent study reported that increased CSF salsolinol levels in normal, treated, and untreated PD patients were not affected by L-DOPA treatment, but may be associated with the occurrence of dementia [90]. The failure to associate the development of PD with increased endogenous isoquinoline derivatives may be due to the possibility that PD patients have a genetically determined susceptibility to normal brain levels of isoquinoline derivatives.

Indeed, metabolism of various xenobiotics (e.g. phenytoin and debrisoquine) may be impaired in PD, and these may be determined genetically [3–5, 86]. In addition, other substances that are acquired (or genetically derived) may be required to operate simultaneously or in sequence with isoquinoline derivatives to cause nigral cell death. This hypothesis relates to the observation that the co-administration of ethanol or acetaldehyde with MPTP in mice markedly increased irreversible dopamine depletion and nigrostriatal damage as compared with MPTP treatment alone [91, 92]. Although the underlying mechanism of this is not known, it is not due to alterations in the metabolism, dopaminergic uptake, or pharmacokinetics of MPTP [91, 92]. The ageing process may also play an important role in the activity of isoquinoline derivatives through increased synthesis and accumulation, reduced ability for metabolism and clearance, and increased mitochondrial susceptibility. However, at present, little or nothing is known of these factors with regards to the neurotoxic potential of isoquinoline derivatives.

CONCLUSION

These early studies have provided valuable insights into some of the neurotoxic properties of isoquinoline derivatives. The emerging evidence suggests that isoquinoline derivatives are metabolized to generate active isoquinolinium cations and free radicals. Isoquinoline derivatives are selective and potent inhibitors of complex I and α-KGDH activity, but are less effective inhibitors of NADH-linked mitochondrial respiration, are moderate to poor substrates for the dopamine re-uptake system, and display weak toxicity to dopamine-containing cells. Substrate affinity for the dopamine re-uptake system appears to be the limiting factor in cytotoxicity of isoquinoline derivatives, although inhibition of mitochondrial function and/or generation of free radicals mediate necrotic and apoptotic cell death. Therefore, it is conceivable that if there are high concentrations of and/or prolonged exposure to isoquinoline derivatives, or if these compounds are formed intraneuronally, isoquinoline derivatives could cause neuronal death in PD. There are, however, other areas of investigation that will further advance our knowledge of the neurotoxic potential of isoquinoline derivatives and their relevance to the aetiology of PD. In particular, it is crucial to perform further studies to determine the normal and pathological (in the pre-symptomatic and symptomatic stages of untreated PD) concentrations of isoquinoline derivatives in brain to provide a clearer picture of their neurotoxic potential for causing PD.

K. St. P. M. is the recipient of a Wellcome Trust Toxicology studentship. P-A. C. and B. T. acknowledge support from the Swiss National Science Foundation. C. A., S. C., and A. C. gratefully acknowledge the Italian Council of Research (CNR, Rome) for financial support. This study was supported by the National Parkinson Foundation (Miami), the Medical Research Council, and the Parkinson's Disease Society.

References

1. Forno S, Pathology of Parkinson's disease. In: *Movement Disorders, Neurology 2*. (Eds. Marsden CD and Fahn S), pp. 21–40. Butterworth Scientific, London, 1981.
2. Agid Y, Javoy-Agid F and Ruberg M, Biochemistry of neurotransmitters in Parkinson's disease. In: *Movement Disorders* (Eds. Marsden CD and Fahn S), Vol. 2, pp. 166–230. Butterworth & Co. Publishers, Stoneham, MA, 1987.
3. Jenner P, Schapira AHV and Marsden CD, New insights into the cause of Parkinson's disease. *Neurology* **42:** 2241–2250, 1992.
4. Steventon GB, Heafield MTE, Waring RH and Williams AC, Xenobiotic metabolism in Parkinson's disease. *Neurology* **39:** 883–887, 1989.
5. Bandmann O, Vaughan J, Holmans P, Marsden CD and Wood NW, Association of slow acetylator genotype for *N*-acteyltransferase 2 with familial Parkinson's disease. *Lancet* **350:** 1136–1139, 1997.
6. Dexter DT, Carayon A, Javoy-Agid F, Agid Y, Wells FR, Daniel SE, Jenner P and Marsden CD, Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. *Brain* **114:** 1953–1975, 1991.
7. Saggu H, Cooksey J, Dexter D, Wells FR, Lees A, Jenner P and Marsden CD, A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. *J Neurochem* **53:** 692–697, 1989.
8. Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, Jenner P and Marsden CD, Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann Neurol* **36:** 348–355, 1994.
9. Sian J, Dexter DT, Lees AJ, Daniel S, Jenner P and Marsden CD, Glutathione-related enzymes in brain in Parkinson's disease. *Ann Neurol* **36:** 356–361, 1994.
10. Dexter DT, Holley AE, Flitter WD, Slater TF, Wells RF, Daniel SE, Lees AJ, Jenner P and Marsden CD, Increased levels of lipid hydroperoxides in the parkinsonian substantia nigra: An HPLC and ESR study. *Mov Disord* **9:** 92–97, 1994.
11. Sanchez-Ramos JR, Overvik E and Ames BN, A marker of oxyradical-mediated DNA damage (8-hydroxy-2′-deoxyguanosine) is increased in nigro-striatum in Parkinson's disease brain. *Neurodegeneration* **3:** 197–204, 1994.
12. Schapira AHV, Mann VM, Cooper JM, Dexter D, Daniel SE, Jenner P, Clark JB and Marsden CD, Anatomic and disease specificity of NADH CoQ_1 reductase (complex I) deficiency in Parkinson's disease. *J Neurochem* **55:** 2142–2145, 1990.
13. Mizuno Y, Matuda S, Yoshino H, Mori H, Hattori N and Ikebe S, An immunohistochemical study on α-ketoglutarate dehydrogenase complex in Parkinson's disease. *Ann Neurol* **35:** 204–210, 1994.
14. Tanner CM, Epidemiological clues to the cause of Parkinson's disease. In: *Movement Disorders* (Eds. Marsden CD and Fahn S), Vol. 3, pp. 124–144. Butterworth-Heinemann Ltd., Oxford, U.K., 1994.
15. Langston JW, Irwin I and Ricaurte GA, Neurotoxins, parkinsonism and Parkinson's disease. *Pharmacol Ther* **32:** 19–49, 1987.
16. Ross RT, Drug-induced parkinsonism and other movement disorders. *Can J Neurol Sci* **17:** 155–162, 1990.
17. Langston JW, Ballard P, Tetrud JW and Irwin I, Chronic parkinsonism in humans due to a product of meperidine-analogue synthesis. *Science* **219:** 979–980, 1983.
18. Irwin I and Langston JW, MPTP and Parkinson's disease. In:

Natural and Synthetic Neurotoxins (Ed. Harvey AL), pp. 225–256. Academic Press, New York, 1993.
19. Chiba K, Trevor AJ and Castagnoli N Jr, Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem Biophys Res Commun* **120:** 574–578, 1984.
20. Javitch JA, D'Amato RJ, Strittmatter SM and Snyder SH, Parkinsonism inducing neurotoxin *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Uptake of the metabolite *N*-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci USA* **82:** 2173–2177, 1985.
21. Ramsay RR and Singer TP, Energy-dependent uptake of *N*-methyl-4-phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, by mitochondria. *J Biol Chem* **261:** 7585–7587, 1986.
22. Di Monte D, Jewell SA, Ekstrom G, Sandy MS and Smith MT, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridine (MPP^+) cause rapid ATP depletion in isolated hepatocytes. *Biochem Biophys Res Commun* **137:** 310–315, 1986.
23. Mizuno Y, Saitoh T and Sone N, Inhibition of mitochondrial α-ketoglutarate dehydrogenase by 1-methyl-4-phenylpyridinium ion. *Biochem Biophys Res Commun* **143:** 971–976, 1987.
24. Cleeter MJW, Cooper JM and Schapira AHV, Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: Evidence for free radical involvement. *J Neurochem* **58:** 786–789, 1992.
25. Ikeda H, Markey CJ and Markey SP, Search for neurotoxins structurally related to 1-methyl-4-phenylpyridine (MPP^+) in the pathogenesis of Parkinson's disease. *Brain Res* **575:** 285–298, 1993.
26. Nagatsu T, Isoquinoline neurotoxins in the brain and Parkinson's disease. *Neurosci Res* **29:** 99–111, 1997.
27. Nagatsu T and Yoshida M, An endogenous substance of the brain, tetrahydroisoquinoline, produces parkinsonism in primates with decreased dopamine, tyrosine hydroxylase and biopterin in the nigrostriatal regions. *Neurosci Lett* **87:** 178–182, 1987.
28. Deitrich R and Erwin V, Biogenic amine-aldehyde condensation products: Tetrahydroisoquinolines and tryptolines (β-carbolines). *Annu Rev Pharmacol Toxicol* **20:** 55–80, 1980.
29. Rommelspacher H, May T and Susilo R, β-Carbolines and tetrahydroisoquinolines: Detection and function in mammals. *Planta Med* **57 (Suppl):** S85–S92, 1991.
30. Holmstedt B, Betacarbolines and tetrahydroisoquinolines: Historical and ethnopharmacological background. In: *Progress in Clinical and Biological Research* (Eds. Bloom F, Barchas J, Sandler M and Usdin E), Vol. 90, pp. 3–13. Alan R. Liss, New York, 1982.
31. Rommelspacher H and Susilo R, Tetrahydroisoquinolines and β-carbolines: Putative natural substances in plants and mammals. In: *Progress in Drug Research* (Eds. Herausgegeben V and Jucker E), Vol. 29, pp. 415–459. Birkhäuser, Basel, 1985.
32. Niwa T, Yoshizumo H, Tatematsu A, Matsuura S and Nagatsu T, Presence of tetrahydroisoquinoline, a parkinsonism-related compound, in foods. *J Chromatogr* **493:** 347–352, 1989.
33. Makino Y, Ohta S, Tachikawa O and Hirobe M, Presence of tetrahydroisoquinoline and 1-methyl-tetrahydroisoquinoline in foods: Compounds related to Parkinson's disease. *Life Sci* **43:** 373–378, 1988.
34. Kohno M, Ohta S and Hirobe M, Tetrahydroisoquinoline and 1-methyl-tetrahydroisoquinoline as novel endogenous amines in rat brain. *Biochem Biophys Res Commun* **140:** 448–454, 1986.
35. Ohta S, Kohno M, Makino Y, Tachikawa O and Hirobe M, Tetrahydroisoquinoline and 1-methyltetrahydroisoquinoline are present in the human brain: Relation to Parkinson's disease. *Biomed Res* **8:** 453–456, 1987.
36. Niwa T, Nakeda N, Yoshizumi H, Tatematsu A, Yoshida M, Dostert P, Naoi M and Nagatsu T, Presence of 2-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and 1,2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, novel endogenous amines, in parkinsonian and normal human brains. *Biochem Biophys Res Commun* **177:** 603–609, 1991.
37. Niwa T, Takeda N, Hashizume Y and Nagatsu T, Presence of tetrahydroisoquinoline and 2-methyl-tetrahydroisoquinoline in parkinsonian and normal human brains. *Biochem Biophys Res Commun* **144:** 1084–1089, 1989.
38. Maruyama W, Nakahara D, Ota M, Takahashi T, Takahashi A, Nagatsu T and Naoi M, *N*-Methylation of dopamine-derived 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, (*R*)-salsolinol, in rat brains: *In vivo* microdialysis study. *J Neurochem* **59:** 395–400, 1992.
39. Maruyama W, Sobue G, Matsubara K, Hashizume Y, Dostert P and Naoi M, A dopaminergic neurotoxin, 1(*R*),2(*N*)-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, *N*-methyl(*R*)salsolinol, and its oxidation product, 1,2(*N*)-dimethyl-6,7-dihydroxyisoquinolinium ion, accumulate in the nigro-striatal system of the human brain. *Neurosci Lett* **223:** 61–64, 1997.
40. Naoi M, Matsurra S, Parvez H, Takahashi T, Hirata Y, Minami M and Nagatsu T, Oxidation of *N*-methyl-1,2,3,4-tetrahydroisoquinoline into the *N*-methyl-isoquinolinium ion by monoamine oxidase. *J Neurochem* **52:** 653–655, 1989.
41. Naoi M, Matsuura S, Takahashi T and Nagatsu T, A *N*-methyltransferase in human brain catalyses *N*-methylation of 1,2,3,4-tetrahydroisoquinoline into *N*-methyl-1,2,3,4-tetrahydroisoquinoline, a precursor of a dopaminergic neurotoxin, *N*-methylisoquinolinium ion. *Biochem Biophys Res Commun* **161:** 1213–1219, 1989.
42. Niwa T, Takeda N, Tatematsu A, Matsuura S, Yoshida M and Nagatsu T, Migration of tetrahydroisoquinoline, a possible parkinsonian neurotoxin, into monkey brain from blood as provided by gas chromatography–mass spectrometry. *J Chromatogr* **452:** 85–91, 1988.
43. Kikuchi K, Nagatsu Y, Makino Y, Mashino T, Ohta S and Hirobe M, Metabolism and penetration through the blood–brain barrier of parkinsonism-related compounds: 1,2,3,4-tetrahydroisoquinoline and 1-methyl-1,2,3,4-tetrahydroisoquinoline. *Drug Metab Dispos* **19:** 257–262, 1991.
44. Kawai H, Makino Y, Hirobe M and Ohta S, Novel endogenous 1,2,3,4-tetrahydroisoquinoline derivatives: Uptake by dopamine transporter and activity to induce parkinsonism. *J Neurochem* **70:** 745–751, 1998.
45. Kotake Y, Tasaki Y, Makino Y, Ohta S and Hirobe M, 1-Benzyl-1,2,3,4-tetrahydoisoquinoline as a parkinsonism-inducing agent: A novel endogenous amine in mouse and parkinsonian CSF. *J Neurochem* **65:** 2633–2638, 1995.
46. Kotake Y, Yoshida M, Ogawa M, Tasaki Y, Hirobe M and Ohta S, Chronic administration of 1-benzyl-1,2,3,4-tetrahydroisoquinoline, an endogenous amine in the brain, induces parkinsonism in a primate. *Neurosci Lett* **217:** 69–71, 1996.
47. Suzuki T, Fujita S, Narimatsu S, Masubuchi Y, Tachibana M, Ohta S and Hirobe M, Cytochrome P450 isoenzymes catalyzing 4-hydroxylation of parkinsonism-related compound 1,2,3,4-tetrahydroisoquinoline in rat liver microsomes. *FASEB J* **6:** 771–776, 1992.
48. Myers RD, Isoquinolines, beta-carbolines and alcohol drinking: Involvement of opioid and dopaminergic mechanisms. *Experientia* **45:** 436–443, 1989.
49. Thull U, Kneubuhler S, Gaillard P, Carrupt P-A, Testa B, Altomare C, Carotti A, Jenner P and McNaught KStP, Inhibition of monoamine oxidase by isoquinoline derivatives. *Biochem Pharmacol* **50:** 869–877, 1995.

50. Suzuki K, Mizuno Y and Yoshida M, Selective inhibition of complex I of the brain electron transport system by tetrahydroisoquinoline. *Biochem Biophys Res Commun* **162:** 1541–1545, 1989.
51. Suzuki K, Mizuno Y, Yasuhiro Y, Nagatsu T and Mitsuo Y, Selective inhibition of complex I by *N*-methylisoquinolinium ion and *N*-methyl-1,2,3,4-tetrahydroisoquinoline in isolated mitochondria prepared from mouse brain. *J Neurol Sci* **109:** 219–223, 1992.
52. McNaught KStP, Altomare C, Cellamare S, Carotti A, Thull U, Carrupt PA, Testa B, Jenner P and Marsden CD, Inhibition of α-ketoglutarate dehydrogenase by isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Neuroreport* **6:** 1105–1108, 1995.
53. McNaught KStP, Thull U, Carrupt PA, Altomare C, Cellamare S, Carotti A, Testa B, Jenner P and Marsden CD, Inhibition of complex I by isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Biochem Pharmacol* **50:** 1903–1911, 1995.
54. Morikawa N, Nakagawa-Hattori Y and Mizuno Y, Effect of dopamine, dimethoxyphenylethylamine, papaverine, and related compounds on mitochondrial respiration and complex I activity. *J Neurochem* **66:** 1174–1181, 1996.
55. Horgan DJ, Singer TP and Cassida J, Studies on the respiratory chain-linked reduced nicotinamide adenine dinucleotide dehydrogenase. *J Biol Chem* **243:** 834–843, 1968.
56. Mizuno Y, Sone N, Suzuki K and Saitoh T, Studies on the toxicity of 1-methyl-4-phenylpyridinium ion (MPP^+) against mitochondria of mouse brain. *J Neurol Sci* **86:** 97–110, 1988.
57. Hoppel CL, Greenblatt D, Kwok HC, Arora PK, Singh MP and Sayre LM, Inhibition of mitochondrial respiration by analogs of 4-phenylpyridine and 1-methyl-4-phenylpyridinium cation (MPP^+), the neurotoxic metabolite of MPTP. *Biochem Biophys Res Commun* **148:** 684–693, 1987.
58. Gluck MR, Youngster SK, Ramsay RR, Singer TP and Nicklas WJ, Studies on the characterization of the inhibitory mechanism of 4′-alkylated 1-methyl-4-phenylpyridinium and phenylpyridine analogues in mitochondria and electron transport particles. *J Neurochem* **63:** 655–661, 1994.
59. Gluck MR, Krueger MJ, Ramsay RR, Sablin SO, Singer TP and Nicklas WJ, Characterization of the inhibitory mechanism of 1-methyl-4-phenylpyridinium and 4-phenylpyridine analogs in inner membrane preparations. *J Biol Chem* **269:** 3167–3174, 1994.
60. Ramsay RR, Salach JI, Dadgar J and Singer TP, Inhibition of mitochondrial NADH dehydrogenase by pyridine derivatives and its possible relation to experimental and idiopathic parkinsonism. *Biochem Biophys Res Commun* **135:** 269–275, 1986.
61. Suzuki K, Mizuno Y and Yoshida M, Inhibition of mitochondrial respiration by 1,2,3,4-tetrahydroisoquinoline-like endogenous alkaloids in mouse brain. *Neurochem Res* **7:** 705–710, 1990.
62. Sayre LM, Wang F, Arora PK, Riachi NJ, Harik SI and Hoppel CL, Dopaminergic neurotoxicity *in vivo* and inhibition of mitochondrial respiration *in vitro* by possible endogenous pyridinium-like substances. *J Neurochem* **57:** 2106–2115, 1991.
63. McNaught KStP, Thull U, Carrupt PA, Altomare C, Cellamare S, Carotti A, Testa B, Jenner P and Marsden CD, Effects of isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on mitochondrial respiration. *Biochem Pharmacol* **51:** 1503–1511, 1996.
64. Seaton TA, Cooper JM and Schapira AHV, Free radical scavengers protect dopaminergic cell lines from apoptosis induced by complex I inhibitors. *Brain Res* **777:** 110–118, 1997.
65. Maruyama W, Dostert P, Matsubara K and Naoi M, *N*-Methyl(*R*)salsolinol produces hydroxyl radicals: Involvement in neurotoxicity. *Free Radic Biol Med* **19:** 67–75, 1995.
66. Maruyama W, Dostert W and Naoi M, Dopamine-derived 1-methyl-6,7-dihydroxyisoquinoline as hydroxy radical promoters and scavengers: *In vivo* and *in vitro* studies. *J Neurochem* **64:** 2635–2643, 1995.
67. Maruyama W, Naoi M, Kasamatsu T, Hashizume Y, Takahashi T, Kohda K and Dostert P, An endogenous dopaminergic neurotoxin, *N*-methyl-(*R*)-salsolinol, induces DNA damage in human dopaminergic neuroblastoma SH-SY5Y cells. *J Neurochem* **69:** 322–329, 1997.
68. Davila JC, Davis P and Acosta D, Changes in glutathione and cellular energy as potential mechanisms of papaverine-induced hepatotoxicity *in vitro*. *Toxicol Appl Pharmacol* **109:** 28–36, 1991.
69. Heikkila RE, Cohen G and Dembiec D, Tetrahydroisoquinoline alkaloids: Uptake by rat brain homogenates and inhibition of catecholamine uptake. *J Pharmacol Exp Ther* **179:** 250–258, 1971.
70. Hirata Y, Minami M, Naoi M and Nagatsu T, Studies on the uptake of *N*-methylisoquinolinium ion into rat striatal slices using high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* **503:** 189–195, 1990.
71. Liptrot J, Holdup D and Phillipson O, 1,2,3,4-Tetrahydro-2-methyl-4,6,7-isoquinolinetriol depletes catecholamines in rat brain. *J Neurochem* **61:** 2199–2206, 1993.
72. McNaught KStP, Thull U, Carrupt PA, Altomare C, Cellamare S, Carotti A, Testa B, Jenner P and Marsden CD, Inhibition of uptake of [^{3}H]dopamine into striatal synaptosomes by isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Biochem Pharmacol* **52:** 29–34, 1996.
73. Niijima K, Araki M, Ogawa M, Suzuki K, Mizuno Y, Nagatsu I, Kimura H, Yoshida M and Nagatsu T, *N*-Methylisoquinolinium ion ($NMIQ^+$) destroys cultured mesencephalic dopamine neurons. *Biogenic Amines* **8:** 61–67, 1991.
74. Nishi K, Mochizuki H, Furukawa Y, Mizuno Y and Yoshida M, Neurotoxic effects of 1-methyl-4-phenylpyridinium (MPP^+) and tetrahydroisoquinoline derivatives on dopaminergic neurons in ventral mesencephalic-striatal co-culture. *Neurodegeneration* **3:** 33–42, 1994.
75. Goto K, Mochizuki H, Hattori T, Nakamura N and Mizuno Y, Neurotoxic effects of papaverine, tetrahydropapaverine and dimethoxyphenylethylamine on dopaminergic neurons in ventral mesencephalic-striatal co-culture. *Brain Res* **754:** 260–268, 1997.
76. Takahashi T, Maruyama W, Deng Y, Dostert P, Nakahara D, Niwa T, Ohta S and Naoi M, Cytotoxicity of endogenous isoquinolines to human dopaminergic neuroblastoma SH-SY5Y cells. *J Neural Transm* **104:** 59–66, 1997.
77. McNaught KStP, Thull U, Carrupt PA, Altomare C, Cellamare S, Carotti A, Testa B, Jenner P and Marsden CD, Toxicity to PC12 cells of isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurosci Lett* **206:** 37–40, 1996.
78. Yoshida M, Niwa T and Nagatsu T, Parkinsonism in monkeys produced by chronic administration of an endogenous substance of the brain, tetrahydroisoquinoline: The behavioural and biochemical changes. *Neurosci Lett* **119:** 109–113, 1990.
79. Yoshida M, Ogawa M, Suzuki K and Nagatsu T, Parkinsonism produced by tetrahydroisoquinoline (TIQ) or the analogues. In: *Advances in Neurology* (Eds. Narabayashi H, Nagatsu T, Yanagisawa N and Mizuno Y), Vol. 60, pp. 207–211. Raven Press, New York, 1993.
80. Ogawa M, Araki M, Nagatsu I, Nagatsu T and Yoshida M, The effect of 1,2,3,4-tetrahydroisoquinoline (TIQ) on mesencephalic dopaminergic neurons in C57BL-6J mice: Immu-

nohistochemical studies-tyrosine hydroxylase. *Biogenic Amines* **6:** 427–436, 1989.
81. Perry TL, Jones K and Hansen S, Tetrahydroisoquinoline lacks dopaminergic nigrostriatal neurotoxicity in mice. *Neurosci Lett* **85:** 101–104, 1988.
82. Moser A, Siebecker F, Nobbe F and Bohme V, Rotational behaviours and neurochemical changes in unilateral *N*-methyl-norsalsolinol and 6-hydroxydopamine lesioned rats. *Exp Brain Res* **112:** 89–95, 1996.
83. Origitano T, Hannigan J and Collins MA, Rat brain salsolinol and blood–brain barrier. *Brain Res* **224:** 446–451, 1981.
84. McNaught KStP, Thull U, Carrupt PA, Altomare C, Cellamare S, Carotti A, Testa B, Jenner P and Marsden CD, Nigral cell loss produced by infusion of isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration* **5:** 265–274, 1996.
85. Cashaw J, Determination of tetrahydropapaveroline in the urine of parkinsonian patients receiving L-dopa-carbidopa (Sinemet) therapy by high performance liquid chromatography. *J Chromatogr* **613:** 267–273, 1993.
86. Kondo I and Kanazawa I, Debrisoquine hydroxylase and Parkinson's disease. In: *Advances in Neurology* (Eds. Narabayashi H, Nagatsu T, Yanagisawa N and Mizuno Y), Vol. 60, pp. 338–342. Raven Press, New York, 1993.
87. Zhang ZX and Román GC, Worldwide occurrence of Parkinson's disease: An updated review. *Neuroepidemiology* **12:** 195–208, 1993.
88. Jenner P and Olanow CW, Pathological evidence for oxidative stress in Parkinson's disease and related degenerative disorders. In: *Neurodegeneration and Neuroprotection in Parkinson's Disease* (Eds. Olanow CW, Jenner P and Youdim M), pp. 23–45. Academic Press, London, 1996.
89. Fahn S and Cohen G, The oxidant stress hypothesis in Parkinson's disease: Evidence supporting it. *Ann Neurol* **32:** 804–812, 1992.
90. Antkiewicz-Michaluk L, Krygowska-Wajs A, Szczudlik A, Romanska I and Vetulani J, Increase in salsolinol in the cerebrospinal fluid of parkinsonian patients is related to dementia: Advantage of a new high-performance liquid chromatography methodology. *Biol Psychiatry* **42:** 514–518, 1997.
91. Zuddas A, Corsini G, Schinelli S, Johannessen JN, Porzio U and Kopin IJ, MPTP treatment combined with ethanol or acetaldehyde selectively destroys dopaminergic neurons in mouse substantia nigra. *Brain Res* **501:** 1–10, 1989.
92. Zuddas A, Corsini G, Schinelli S, Barker JL, Kopin IJ and Porzio U, Acetaldehyde directly enhances MPP^+ neurotoxicity and delays its elimination from the striatum. *Brain Res* **501:** 11–22, 1989.