Review Letter

Mechanism of the neurotoxicity of MPTP

An update

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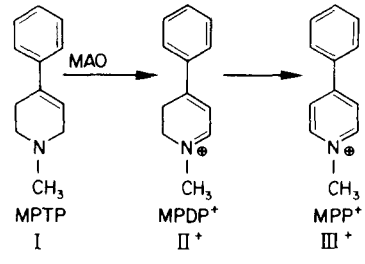
Received 23 August 1990

This review summarizes advances in our understanding of the biochemical events which underlie the remarkable neurotoxic action of MPTP (1-methyl-4-phenyl-1-1,2,3,6-tetrahydropyridine) and the parkinsonian symptoms it causes in primates. The initial biochemical event is a two-step oxidation by monoamine oxidase B in glial cells to MPP^+ (1-methyl-4-phenylpyridinium). A large number of MPTP analogs substituted in the aromatic (but not in the pyridine) ring are also oxidized by monoamine oxidase A or B, is in some cases faster than any previously recognized substrate. Alkyl substitution at the 2'-position changes MPTP, a predominantly B type substrate, to an A substrate. Following concentration in the dopamine neurons by the synaptic system, which has a high affinity for the carrier, MPP^+ and its positively charged neurotoxic analogs are further concentrated by the electrical gradient of the inner membrane and then more slowly penetrate the hydrophobic reaction site on NADH dehydrogenase. Both of the latter events are accelerated by the tetraphenylboron anion, which forms ion pairs with MPP^+ and its analogs. Mitochondrial damage is now widely accepted as the primary cause of the MPTP induced death of the nigrostriatal cells. The molecular target of MPP^+, its neurotoxic product, is NADH dehydrogenase. Recent experiments suggest that the binding site is at or near the combining site of the classical respiratory inhibitors, rotenone and piericidin A.

Monoamine oxidase; MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); Neurotoxin; NADH dehydrogenase

1. INTRODUCTION

In an earlier Trends Biochem. Sci. review [1] we summarized how the sudden appearance of parkinsonism in relatively young patients in the San Francisco Bay Area in 1982 was traced to their use of a designer drug, 'new heroin', manufactured by a clandestine laboratory, and to the MPTP contained in defective batches of the street drug. Almost immediately following the reports that MPTP is neurotoxic to humans [2], subhuman primates and mice [3-5], eliciting damage to dopaminergic neurons, it was shown that MPP^+ (1-methyl-4-phenylpyridinium) is the neurotoxic form and that it arises by 4-e oxidation of MPTP in brain mitochondria [6]. Proof that the enzyme responsible for processing MPTP is monoamine oxidase (MAO B) in the glial cells, of which it is an excellent substrate and that MAO A also oxidizes MPTP, albeit more slowly, was reported soon thereafter [7]. Deprenyl, a selective, mechanism-based inhibitor of MAO B, prevented the oxidation of MPTP by pure MAO B and protected experimental animals from the neurotoxic action of MPTP.



The biochemical events which follow the passage of MPTP through the blood-brain barrier, as visualized in 1987, are summarized in Scheme 1. The first step in the development of neurotoxicity is thus oxidation by MAO B in the astrocytes of the brain to the dihydropyridinium form ($MPDP^+$), which is then further oxidized in part by this enzyme, in part nonenzymically to the pyridinium form, MPP^+ [8]. The MPP^+ is selectively taken up by the dopamine reuptake system at dopaminergic synapses and probably pumped into the stroma of the neurons [9]. MPP^+ is then concentrated from the cytoplasm into the mitochondrial matrix by the electrochemical gradient of the inner

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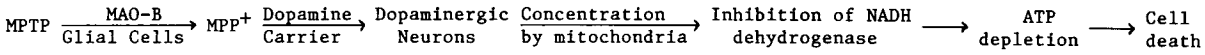
00145793/90/$3.50

Scheme 1



Scheme 1. Steps in the expression of the neurotoxicity of MPTP.

membrane, where it combines with NADH dehydrogenase, probably at the dehydrogenase-Q junction, blocking thereby electron transport [10–12]. This leads to cessation of ATP synthesis, nigrostriatal cell death, and the consequent neurological symptoms characteristic of parkinsonian patients. Some questions remained, such as whether MPP^+ was the only or even the main neurotoxic product arising from MPTP, why there were remarkable differences in species sensitivity to MPTP, or why organs other than the brain are not damaged by MPTP. However, most of the debate centered around the 'mitochondrial hypothesis', namely that, despite their dissimilar structures, MPP^+ acts like rotenone to inhibit mitochondrial respiration versus the 'oxidative stress' hypothesis, which assumes that the effects of hydroxyl and superoxide radicals caused nigrostriatal cell death. The ultimate question, which remains open, is whether the similarity in neuronal lesions and neurological symptoms elicited by MPTP and its congeners and those seen in idiopathic parkinsonism suggests that evironmental compounds of related structure may be causative agents in human parkinsonism [13–14]. The present review summarizes recent advances in these areas and other advances of interest to the biochemical community.

2. BIOACTIVATION

The first step in the formation of neurotoxic products from MPTP is oxidation by monoamine oxidase (MAO) B in the brain. Studies during the past 3 years have established that several neurotoxic MPTP analogs are also rapidly oxidized by MAO A, while yet others are processed almost entirely by the A form (Table I). The specificity pattern shown in Table I for the pure enzymes [15] holds equally well for intact brain mitochondria [16]. It is seen that substitution at 2′ in the aromatic ring greatly enhances the rate of oxidation by MAO A: the longer the side chain, the better substrate it becomes. In contrast, oxidation by MAO B progressively decreases with the length of the alkyl substituent beyond 2′-methyl-MPTP. In accord with this, while the toxicity of MPTP to sensitive species is completely prevented by selective inhibitors of the B enzyme, such as deprenyl, protection from 2′-methyl MPTP requires both MAO A and B inhibitors (clorgyline and deprenyl), and clorgyline alone protects from the neurotoxic effects of 2′-ethyl MPTP. This gratifying agreement between biochemical and pharmacological studies suggests the generalization that in order for a tetrahydropyridine to be neurotoxic, it must be oxidized by either MAO A or B.

The mechanism by which $MPDP^+$, the primary oxidation product of MPTP, is further oxidized to MPP^+ in the cell remains uncertain. At relatively high concentrations and alkaline pH, $MPDP^+$ disproportionates to MPP^+ and MPTP, but the reaction is not likely to be significant at the intracellular concentrations prevailing in MPTP-treated animals. Slow non-enzymic oxidation by molecular O_2 has also been observed. Both MAO A and B were shown [8] to oxidize $MPDP^+$ and its analogs relatively slowly, the rate in each case being two orders of magnitude less than the values shown in Table I. In brain mitochondria the oxidation is sensitive to MAO B inhibitors, but it has been reported to be insensitive in hepatocytes [17], perhaps because of the

Table I

Oxidation of MPTP and its analogs by pure MAO

Substrate	Turnover number/K_m	
	MAO A	MAO B
Control	1283	1064
MPTP	143	523
2′-Methyl-MPTP	593	1275
2′-Ethyl-MPTP	688	295
2′-*n*-Propyl-MPTP	658	86
2′-Methoxy-MPTP	511	233
2′-Isopropyl-MPTP	1131	51
2′,6′-Dimethyl-MPTP	490	209
3′-Methyl-MPTP	76	650
2′-Fluoro-MPTP	100	1054
2′-Chloro-MPTP	400	1353
3′-Fluoro-MPTP	391	900
3′-Chloro-MPTP	567	1132
3′-Bromo-MPTP	300	2036
3′-Methoxy-MPTP	–	944
4′-Methyl-MPTP	58	345
4′-Fluoro-MPTP	–	423
4′-Chloro-MPTP	69	595
4′-Amino-MPTP	12	54
4′-Nitro-MPTP	185	16
EPTP	28	200
MCTP	270	688
M(4Bz)TP	100	2675
M(4tBu)TP	–	92
PPTP	73	31
2′-Trifluoromethyl-MPTP	169	520
3′-Trifluoromethyl-MPTP	214	514
MTHIQ*	0	0
1-methyl-4(2-pyridinyl)-TP	19	41
1-methyl-4(1-CH_3-2-pyrroyl)-TP	534	347

The control substrates were kynuramine for MAO-A and benzylamine for MAO-B. Although this isoquinoline is not oxidized, it is a competitive inhibitor of both enzymes.

numerous catabolic reactions occuring in liver but not in brain.

3. CONTRIBUTIONS TO THE BIOCHEMISTRY OF MONOAMINE OXIDASES

Perhaps the most significant new information derived from these studies concerns the substrate specificities of MAO A and B. Several laboratories have studied the oxidation of synthetic analogs of MPTP [18–20]. As seen in Table I, 2′-methyl-MPTP, 2′-Cl-MPTP, 3′-Cl-MPTP, and 1-methyl-4-benzyl-tetrahydropyridine are all better substrates of MAO B than benzylamine, previously the best substrate known. In the case of MAO A, 2′-isopropyl-MPTP is nearly as good a substrate as kynuramine, previously believed to be its most rapidly oxidized substrate, while the turnover number of 2′-*n*-propyl-MPTP (250 at 30°C) is considerably higher than that of kynuramine (154), although the ratio of turnover number/K_m favors kynuramine because of its lower K_m value. Other studies have shown that 2′-F-MPTP, 2′-CF_3-MPTP, and 3′-CF_3-MPTP are excellent substrates of MAO B but not of A, while 1-CH_3-4(1-CH_3-2-pyrroyl)-tetrahydropyridine is a good substrate of both forms.

In addition to irreversible inactivation, MAO A and B are reversibly inhibited by the products of MPTP oxidation ($MPDP^+$ and MPP^+ [21]. Subsequent studies on a large series of MPP^+ analogs showed that, as in the case of MPP^+, the A enzyme is far more sensitive to reversible inhibition than the B form [15]. Very recent studies [22] demonstrated that 4′-alkyl substituted MPP^+ analogs are particularly effective competitive inhibitors of MAO A. Thus, the K_i values (μM) for MAO at 30°C are: MPP^+ (3.0), 4′-methyl-MPP^+ (0.2), 4′-*n*-pentyl-MPP^+ (0.13), 4′-*n*-heptyl-MPP^+ (0.59), 4′-*n*-pentyl-azido-MPP^+ (0.075). The corresponding K_i values for MAO B range from 32 to 230 μM.

Plasma amine oxidase has been reported [23] not to oxidize MPTP or 16 of its analogs tested, although most of them are moderately good reversible inhibitors of the enzyme.

The serendipitous circumstance that $MPDP^+$, the product of the first 2-e oxidation of MPTP, is chemically stable, in contrast to the imines formed from primary and secondary amines, has permitted reexamination of the kinetic mechanism of MAO B [24]. The widely held concept that flavoprotein oxidases, including MAO B, operate either by a binary or a ternary complex mechanism, depending on the substrate, had to be modified, because the enzyme can operate by three alternate mechanisms, a binary complex, and two ternary complexes of reduced enzyme-O_2-product, and reduced enzyme-O_2-substrate and these are not mutually exclusive but may occur concurrently. Recently, extension of this work to MAO A revealed that it operates by both a binary complex (ping-pong) mechanism and by a ternary complex involving reduced enzyme-substrate-O_2, not product (MPP^+) that leads only to formation of a dead-end complex [25].

The fact that MPTP and its analogs undergo two 2-e oxidation steps by MAO A and B, each of which is accompanied by mechanism-based inactivation, has offered a unique opportunity to explore the characteristics of mechanism-based inactivation of enzymes in sequential reactions. Early studies [8] indicated that the rate of inactivation of MAO B by MPTP and $MPDP^+$ were identical, suggesting that processing of the latter compound was responsible for the irreversible loss of activity. Recent studies [26], using a rapid scan spectrophotometer and a multicomponent analysis program, confirmed that the mechanism-based inactivation of MAO B by the dihydropyridinium is faster than inactivation by the tetrahydropyridinium for MPTP and several analogs, whereas the reverse is true of MAO A, which processes $MPDP^+$ and its analogs poorly. Ottoboni et al. [27] also came to the conclusion that the mechanism-based inactivation of MAO B in the course of MPTP oxidation is primarily due to the second 2-e oxidation. These authors also concluded, on the basis of kinetic deuterium isotope effect measurements, that the rate determining step in MPTP oxidation by MAO B is C–H cleavage and thus its mechanism more likely involves a hydrogen atom abstraction than deprotonation of an iminium radical.

4. IS MPP^+ NEUROTOXIC PRODUCT OF MPTP METABOLISM?

Soon after the discovery that MPTP is metabolized to $MPDP^+$ and MPP^+ in the brain, several lines of evidence pointed to MPP^+ being the main neurotoxic product. Perhaps the most persuasive evidence was the high affinity of MPP^+ (but not of $MPDP^+$) for the dopamine carrier (dopamine reuptake system), the protection from the neurotoxicity of MPTP by mazindol [9,28], a specific inhibitor of this carrier, and the selective toxicity of MPP^+ to dopaminergic neurons in culture [29]. Castagnoli's group [30] also considered that $MPDP^+$ or a ring-opened form of it might alkylate proteins and contribute to neurotoxicity. Recent studies [31], however, ruled out covalent binding of MPTP metabolites in vivo as relevant to toxicity. At this writing, then, consensus seems to have been reached implicating MPP^+ as the primary neurotoxic form.

5. THE OXIDATIVE STRESS HYPOTHESIS

One of the two main hypotheses for the ultimate cause of the destruction of dopaminergic neurons by MPP^+ held that superoxide and hydroxyl radicals generated during intracellular reduction and reoxida-

tion of MPP^+ were responsible, akin to the action of the herbicide, Paraquat. Although the comparison was inappropriate, because the low redox potential of MPP^+ (−1.07 V) precludes reduction in the cell, the hypothesis remains popular with some biologists [32]. However, there is evidence that, in contrast to MPTP induced parkinsonism, the idiopathic disease due to the progressive decline in the number of nigrostriatal neurons on aging is due to the free radicals produced in the MAO B-catalyzed oxidation of dopamine in aged animals, which have increased levels of MAO B, owing to the proliferation of glial cells [33,34]. Evidence supporting the oxidative stress hypothesis has always been indirect; e.g. that MPTP lowers glutathione in dopamine neurons, or that glutathione and antioxidants protect from the neurotoxicity of MPTP. These reports could not be confirmed in subsequent studies [35,36]. Perhaps the most decisive evidence against the oxidative stress hypothesis has come from the demonstration [37] that anti-oxidants do not protect dopamine neurons in culture from MPP^+.

6. THE MITOCHONDRIAL HYPOTHESIS

The hypothesis that the neurotoxic effect of MPP^+ is the result of shutting off mitochondrial respiration and, hence, oxidative phosphorylation by inhibiting NADH dehydrogenase at the same site as rotenone and barbiturates [38], has received impressive support in recent years. Using dissociated dopamine neurons in culture, Sanchez-Ramos et al. have demonstrated that the barbiturate pentothal protects dopamine neurons in culture against MPP^+ [37]. Moreover, MPP^+ caused extensive oxidation of cytochrome *b* in rat striatal slices, as expected if the toxin interrupted electron flux from NADH dehydrogenase to Q. More recent evidence has come from the finding of mitochondrial abnormalities in the nigral neurons of MPTP treated monkeys [39] and Complex I deficiency in the nigrostriatum of parkinsonism patients [40,41]. The fact that MPP^+ and its analogs compete with the respiratory chain inhibitors rotenone and piericidin for binding to NADH dehydrogenase (cf. section 9) provides further, strong support for this hypothesis.

7. FROM ACCUMULATION IN MITOCHONDRIA TO CELL DEATH

In order for MPP^+ to inhibit NADH dehydrogenase extensively, it must be concentrated from the estimated 30 μM cytoplasmic concentration seen in MPTP-treated rodents to millimolar levels in the mitochondria [12]. This concentration is rapidly reached in response to the electrical gradient across the membrane. Some characteristics of the uptake initially suggested that the process might be carrier-mediated [1], but studies using charged and uncharged structural analogs indicated passive Nernstian transport, as for other lipophilic cations [42]. Interpretation of the process was further complicated by the fact that various MPP^+ analogs accumulated to very different levels in the mitochondrion, depending on their structures [43].

The confusion was resolved by the demonstration that the lipophilic anion, tetraphenylboron (TPB^-), which carries cations into hydrophobic environments by ion-pairing, dramatically enhances the inhibition of mitochondrial respiration by MPP^+ [44–46]. The remarkable potentiation of MPP^+ toxicity to mitochondria by trace amounts of TPB^- (up to 1000-fold with some MPP^+ analogs) is the result of at least two distinct processes (Scheme 2). TPB^- accelerates the passage of MPP^+ across the inner membrane and raises the matrix concentration 2–4-fold. However, this does not fully account for the 10^2–10^3-fold lowering of the IC_{50} value for the inhibition of mitochondrial respiration. The second effect was noted [41,45] in studies of inverted submitochondrial particles, in which the rotenone site of NADH dehydrogenase is exposed to the cytoplasm. In such preparations TPB^- potentiated the inhibitory effect of MPP^+ by one order of magnitude and that of some MPP^+ analogs by 2 orders of magnitude. This suggests that ion pairing between MPP^+ and TPB^- helps the penetration into the hydrophobic pocket on the dehydrogenase where MPP^+, rotenone, and barbiturates are thought to combine. The neutral 4-phenylpyridine molecule, although a much better inhibitor than MPP^+ in submitochondrial particles, is a poor inhibitor in intact mitochondria (because it is not concentrated by the electrical gradient) and its effect is not potentiated by TPB^-. Moreover, 4′-heptyl-MPP^+, a hydrophobic molecule, is ≈1000 times more inhibitory than MPP^+ in submitochondrial particles, but TPB^- does not potentiate its action, perhaps because it can penetrate the hydrophobic site unaided owing to its pronounced lipophilicity.

8. STRUCTURAL REQUIREMENTS FOR INHIBITION

If the inhibition of State 3 respiration in mitochondria by MPP^+ is due to a specific block between the highest potential Fe–S cluster of NADH dehydrogenase and Q (Fig. 1), then all MPP^+ analogs which are good inhibitors of respiration in mitochondria should also be effective inhibitors of NADH oxidation in inverted membranes. With over 20 MPP^+ analogs tested, this was true in each case [43]. Thus, 4′-methyl-MPP^+ is much more potent than MPP^+ in mitochondria; its IC_{50} value for inhibiting NADH oxidase activity in submitochondrial particles (ETP) is 10 times lower than that of MPP^+. The 4′-methyl, 4′-*n*-propyl, 4′-*n*-pentyl, and 4′-heptyl analogs are increasingly more potent in both systems.

Scheme 2

Cytoplasmic MPP^+ (20 to 30 μM)	Concentration by electrochemical Gradient	Intramitochondrial MPP^+ (>10 mM)	Penetration into hydrophobic binding site	Inhibition of NADH dehydrogenase
	Step 1		Step 2	

Scheme 2. Fate of MPP^+ after being pumped into the neuron. Both steps are accelerated by TPB^-.

There are a number of pyridines, however, which are good inhibitors in submitochondrial particles but not in intact mitochondria. The familiar example is 4-phenylpyridine, as discussed above. MPTP, 4-phenylpiperidine, and other uncharged analogs behave similarly. Measurement of the accumulation of labeled MPP^+ analogs by the mitochondria explains in the majority of cases the lesser effectiveness of a compound in inhibiting mitochondrial respiration [43]. An exception to this is 1,1-dimethyl-4-phenyltetrahydropyridinium, a reasonably good inhibitor in submitochondrial particles, appreciably but slowly concentrated in the mitochondria, which, nevertheless, has no measurable effect on mitochondrial respiration after a 5 min incubation [43]. The reason in this case has been shown to be the cumulative effect of slow penetration both into the mitochondria and, particularly, to the hydrophobic binding site on NADH dehydrogenase, since the presence of 10 μM TPB^- greatly enhances its inhibitory effect in submitochondrial particles and brings about a dramatic rise in its toxicity to mitochondrial respiration. Thus, for an MPP^+ analog to be a good inhibitor of NAD-linked oxidations in mitochondria, it must be positively charged at neutral pH, concentrated by the electrical gradient and able to penetrate the hydrophobic rotenone site on NADH dehydrogenase.

9. IDENTIFICATION OF THE BINDING SITE ON NADH DEHYDROGENASE

When EPR studies [47] suggested that the target of MPP^+ is between the highest potential Fe-S cluster of NADH dehydrogenase and Q, as for rotenone, barbiturates, and piericidin A, the exciting possibility arose that all these inhibitors are bound at or near the same site. With the advent of MPP^+ analogs with very low K_i values for NADH dehydrogenase, such as the 4′-alkyl derivatives, direct testing of this hypothesis became possible. Thus, the K_i values range from 680 μM for 4′-methyl-MPP^+ to 4 μM for 4′-*n*-decyl-MPP^+, the latter approaching the K_i for rotenone. All but the *n*-heptyl and *n*-decyl analog nevertheless dissociate on dilution, so that they may be completely removed by centrifugation and washing of submitochondrial particles, with resultant recovery of the activity, whereas piericidin A remains firmly (but non-covalently) bound at the specific site during such treatment, and rotenone dissociates only partly during several cycles of centrifugation and resuspension in sucrose-serum albumin. This difference in the dissociation of the enzyme-inhibitor complexes permitted the demonstration that MPP^+ analogs prevent the binding of rotenone and of piericidin A to NADH dehydrogenase and thus protect the enzyme from their powerful inhibitory effect [48,49]. Fig. 2 shows that, at a low concentration of piericidin prior to treatment with 4′-methyl, 4′-*n*-propyl, and 4′-*n*-pentyl-MPP^+ effectively protects the enzyme from inhibition by piericidin A and *t*-butyl-MPP^+ (1 mM) completely prevents the inhibition. In Tabel III, [^{14}C]piericidin A was used to illustrate the fact that the prevention of piericidin binding parallels protection from piericidin inhibition MPP^+ and

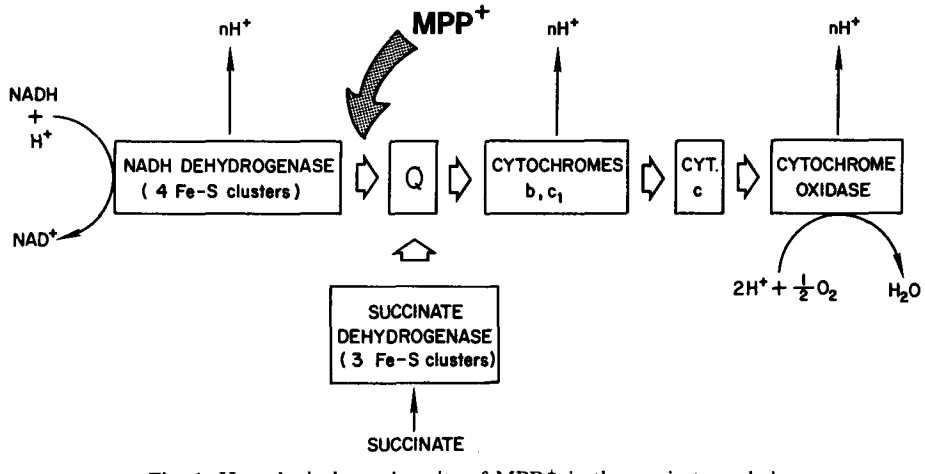


Fig. 1. Hypothetical reaction site of MPP^+ in the respiratory chain.

Table II

Inhibition of NADH oxidase activity in submitochondrial particles by 4'-alkyl analogs of $MPTP^+$

Substituent	IC_{50} (μM)	
	$-TPB^-$	$+TPB^-$
H	5000	700
4'-methyl	680	190
4'-propyl	200	30
4'-*t*-butyl	140	3
4'-pentyl	45	12
4'-heptyl	6	6
4'-decyl	4	4

ETP (2 mg/ml) was preincubated for 5 min at 30°C with various concentrations of the inhibitor. TPB^- (10 μM) was added to enhance penetration of the analog into the hydrophobic site.

various 4'-alkyl analogues. Similar results were obtained using 4'-alkyl-MPP^+ analogs to protect NADH dehydrogenase from inhibition by and binding of rotenone [48,49].

A logical extension of these studies, now in progress in our laboratory, will be the identification of the peptide involved in binding these inhibitors, using photoaffinity labeling with 4'-azido-^{3}[H]MPP^+.

10. ENDOGENOUS NEUROTOXINS RELATED TO MPTP

Continued interest in the biochemistry of MPTP has also triggered an ongoing search for structurally related, endogenous compounds which might be involved in the etiology of parkinsonism. Recent studies in Japan have concentrated on isoquinolines. Thus, 1, 2, 3, 4-tetrahydroisoquinoline (TIQ) and its 1-methyl derivative (1-Me-TIQ) have been detected in human [50] and rat [51] brain. TIQ has been reported to cause parkinsonism in primates, and to inhibit NADH dehydrogenase and tyrosine decarboxylase (involved in the synthesis of dopamine) in rodent brain preparations. Since TIQ is widely distributed in foods, it may not be an endogenous compound. Naoi et al. [52] suggest that the 1-methylisoquinolinium ion is the toxic form, derived from TIQ by methylation via *N*-methyltransferase to 1-Me-TIQ, followed by MAO-catalyzed oxidation. In our hands, however, 1-Me-TIQ is not oxidized by pure MAO A or B or by brain mitochondria. Moreover, if the activity of *N*-methyltransferase in the brain were sufficient for the role postulated above, one wonders why 4-phenylpyridine is not neurotoxic by virtue of methylation to MPP^+.

Methylation of metabolic derivatives of indoleamines and tryptophan yields B-carbolines which resemble MPP^+ with a nitrogen bridge between the two rings. Acute administration of these compounds produced behavior changes and decreased dopamine levels in monkeys, but not persistent parkinsonism. 2-Methyl harmine is as toxic as MPP^+ to cells in culture [53], so may well be a candidate for long-term effects.

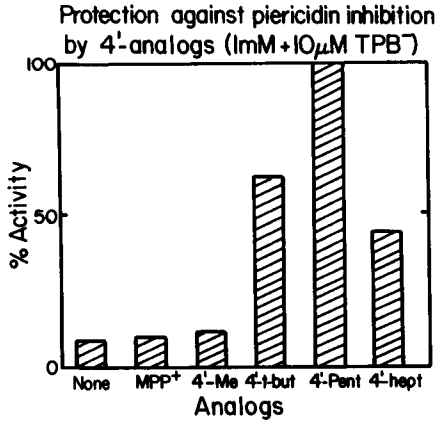


Fig. 2 Protection of NADH dehydrogenase against piericidin inhibition by 4'-alkyl-MPP^+ analogs. The experiment was conducted by incubation submitochondrial particles (ETP) with 0.4 μM piericidin with or without 1 mM MPP^+ analogs and 10 μM TPB^-. The latter served to increase penetration of the positively charged analogs into the target site. The suspension was then washed by centrifugation with 2% bovine serum albumin (BSA)/0.25 M sucrose/0.25 M phosphate, pH 7.4, so as to remove spuriously bound piericidin as well as the 4'-alkyl-MPP^+ analogs. Under these conditions all MPP^+ analogs are removed, except for 4'-heptyl-MPP^+, which is incompletely washed out.

11. RELATION TO IDIOPATHIC PARKINSONISM

While some differences have been observed in the histopathology of idiopathic parkinsonism and the MPTP-induced disease in primates, the similarities are more impressive than the differences. The signs of a chronic disease which develops over a period of years or decades might be expected to differ from those rapidly appearing after MPTP injection. Few would deny that MPTP may not only provide a useful model for the human disease but might also yield clues to the identity of environmental xenobiotics related to the etiology of parkinsonism. At this point it seems clear that for an environmental tetrahydropyridine to be acutely neurotoxic, it must be oxidized by MAO A or B, pumped into the dopamine neurons by the carrier, and inhibit mitochondrial respiration on NAD-linked substrates. If one of these requirements is not met but the compound inhibits NADH oxidase, it might still be a chronic, cumulative neurotoxin, as has been proposed for 4-phenylpyridine.

The exciting studies with MPTP world-wide have had another, more immediate benefit for the treatment of parkinsonism in that they stimulated clinical trials of selegiline (deprenyl) in the USA and its therapeutic use [54]. Building on earlier observations in Europe that the use of this MAO B inhibitor in conjunction with L-DOPA prolongs the life of parkinsonian patients, well-

Table III

Protection of NADH dehydrogenase by 4'-alkyl-MPP$^+$ analogs from piericidin binding and inhibition

Analog	Concentration (mM)	Activity remaining	Protection (%)	Bound inhibitor remaining	Protection
None		7.14	0	1.66	0
4'-Me-MPP$^+$	10	46	42	0.99	40
4'-Pr-MPP$^+$	1.5	68	66	0.61	63
4'-Pe-MPP$^+$	0.45	75	73	1.25	75
4'-*t*-Bu-MPP$^+$	0.06	16	10	1.31	21
4'-hept-MPP$^+$	0.03	3.6	0	1.6	0

The experiment was carried out as in Fig. 2, except that [^{14}C]piericidin was used at 0.52 μM concentration and the concentration of the MPP$^+$ analogs was varied. TPB$^-$ was present at 10 μM concentration. The results represent [^{14}C]piericidin and activity remaining after 3 washes of the particles with 2% BSA/0.25 M sucrose/0.025 M phosphate, pH 7.4. NADH oxidase activity and piericidin binding are in arbitrary units.

controlled studies with early parkinsonian patients provided highly significant data demonstrating that selegiline alone delays the progress of the disease and the need for L-DOPA treatment.

12. SOME UNRESOLVED PROBLEMS

Despite the impressive advances in understanding of the events which follow the systemic administration of MPTP, a number of interesting questions remain unanswered. Some of the seemingly conflicting reports result from extrapolating experiments on one organ or one type of cell to a very different one, or to arguing from chemical experiments conducted under conditions far from those likely to occur in the cell to what might happen in vivo.

The variety of detoxifying reactions occurring in other organs but absent from neuronal tissue has often been invoked as one explanation for the resistance of cells from liver, kidney etc., to irreversible damage by MPTP in vivo. While the degree to which cytochrome P-450 and flavin containing monooxygenase detoxify MPTP in such tissues is uncertain [55,56], it is clear that MPP$^+$ accumulation is far less extensive than in neurons. The rapid removal of the toxic MPP$^+$ from the circulation in part by sequestration in adrenal chromaffin vesicles, seems to provide additional protection [57]. Moreover, unlike neurons, other cells may survive mitochondrial injury by their ability to form ATP anaerobically and to regenerate. There is no question, however, that in vitro even hepatocytes can be killed by MPTP under appropriate conditions. The most important reason for the selective effect by MPTP in killing dopamine neurons still seems to be the action of the dopamine carrier which concentrates MPP$^+$ in these neurons.

A related question is the reason for the differences in susceptibility of different species to irreversible damage by MPTP. The hypothesis remains viable that the most susceptible species (man, subhuman primates, C57 black mice) contain neuromelanin, which binds MPP$^+$ and may serve as a depot for it, whereas rats, and other resistant species lack it. Another interesting hypothesis implicates the high MAO B activity of the capillary wall in rats, in contrast to species sensitive to MPTP. A protective effect could come from oxidation of MPTP at the blood-brain barrier, so that the charged product cannot pass. The possibility that the resistance of rats to irreversible neuronal damage by MPTP may be a function of slow penetration into the brain is compatible with the finding that the stereotaxic administration of MPP$^+$ to the nigrostriatum of rats results in lactate accumulation and other evidence of mitochondrial injury [58]

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