acetone in mobile phase were calculated by the least-squares method from the R_m values in the linearity range of the curves, which were plotted as R_m values versus acetone concentrations.

Solubilization Study. A solution of 2-(hydroxypropyl)-β-cyclodextrin was prepared by dissolving 5 g of the modified starch into 10 mL of cold, degassed, deionized water which had been adjusted to pH 7 with sodium hydrogen carbonate. An excess of the selected tryptophan-CDS was then suspended in the solution and the system was sonicated at 0 °C for 1 h. The mixture was then filtered through 0.45 μm polyvinylidene difluoride membranes and the filtrate frozen on liquid nitrogen. The solid was lyophilized and milled to give a powder which passed through a 60 mesh sieve. Drug incorporation was calculated by UV spectroscopy. Standard curves for the tryptophan derivatives were prepared in methanol and were linear ($r^2 > 0.999$) over the concentration range examined.

Activity Study. Induction of Hypertension in Animals. The antihypertensive activity of tryptophan-CDS compounds was assessed by monitoring changes in systolic blood pressure (BP). To elevate BP, male Sprague-Dawley rats (Blue Spruce Farms), weighing 200–250 g, were administered pentobarbital at 40 mg/kg (ip) and unilaterally (left) nephrectomized. Animals were then implanted with either single 50-mm Silastic tube (Dow Corning; 0.058 in. i.d. and 0.077 in. o.d.) containing deoxycorticosterone acetate (DOCA) or an empty tube (shunt control). After DOCA implantation, blood pressures were monitored weekly by a tail cuff procedure for at least 4 weeks until systolic pressures were ≥150 mmHg, which is an increase of approximately 40 to 50 mmHg.[44] Animals were placed into a 30 °C chamber for 10–15 min to warm the rat to ensure efficient BP recordings. A pressure cuff was then placed at the base of the tail and seven determinations were recorded on a physiograph and averaged. Rats were then returned to their home cages until the next BP recording.

Drug Administration. After obtaining a baseline (0 h) blood pressure, rats (6 per group) received a single tail vein injection of either DL-tryptophan (1; 40.35 mmol/kg), CDSs **5a**, **5b**, **5c**, or ethyl 1-methyl-1,4-dihydronicotinate at doses equimolar to 1. The drug vehicle, 2-(hydroxypropyl)-β-cyclodextrin (HPCD; 40% [w/v]) was administered at a volume of 1 mL/kg. Blood pressures were measured at 1, 2, 3, 4, and 24 h after injection.

Statistical Treatment of Results. The significance of differences among mean values was determined by analysis of variance (ANOVA) and Student-Newman-Keuls tests.[45] The level of probability for all tests was $P < 0.05$.

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In Vivo Intracerebral Microdialysis Studies in Rats of MPP^+ Analogues and Related Charged Species

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The in vivo dopaminergic neurotoxic properties of 45 MPTP and MPP^+ analogues and related compounds were examined by an intrastriatal microdialysis assay in conscious rats. MPP^+-like toxicity, as evidenced by the irreversible effects on DA release and enhancement of lactate formation, was observed with a variety of structural types although no compound was more toxic than MPP^+. The following global structure–toxicity relationships could be derived: (1) only permanently charged compounds showed neurotoxic effects; (2) with the exception of amino groups, hydrophilic substituents abolished toxicity; (3) activity was enhanced by lipophilic groups although increased steric bulk around the nitrogen atom tended to decrease activity; (4) nonaromatic, quaternary systems (methiodide of MPTP, guanidinium derivatives) were only weakly toxic; and (5) certain bi- and tricyclic systems, including putative metabolites of potential endogenous MPTP-like compounds, were weakly toxic. The lack of toxic effects following perfusions with DA itself confirmed that MPTP dopaminergic neurotoxicity is not likely to be mediated by the MPP^+-induced release of DA. With some interesting exceptions, these in vivo data correlate reasonably well with in vitro data on the nerve terminal uptake properties and the inhibitory effects on mitochondrial respiration of these compounds.

Introduction

An understanding of structure–toxicity relationships of compounds related to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (**MPTP, 45**) is important for the identification of potential environmental and endogenous neurotoxins and may contribute to the elucidation of the mechanism of action of MPTP. Most in vivo studies have been performed with tetrahydropyridine derivatives[1-3] which, by analogy with the monoamine oxidase (MAO) B

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bioactivation of MPTP to the 1-methyl-4-phenylpyridinium species MPP^+ (1),[4-7] must be metabolized to a pyridinium species. Although few MPTP analogues appear to be good MAO substrates, chronic exposure to low levels of neurotoxic pyridinium derivatives formed through the slow oxidation of poor MAO substrates or substrates of other oxidases may contribute to neurodegenerative processes.[8] In addition, consideration must be given to the possible access to the central nervous system of neurotoxic lipophilic pyridinium and related quaternary compounds.

Attempts to assess the neurotoxic potential of pyridinium and related species have relied on in vitro measurements of their substrate properties for the dopamine (DA) transporter[9] and their inhibitory properties on mitochondrial respiration,[9,10] two characteristics of MPP^+. Recently the in vitro toxicity of a variety of potential neurotoxins has also been studied in cultured embryonic rat dopaminergic neurons.[11] In vivo studies have been performed by intranigral application of MPP^+ in rats followed by measurement of the striatal dopamine content several weeks later.[12a] This paradigm has also been used for some MPP^+ derivatives[12b] and recently for a β-carbolinium analogue.[12c] We have now extended such in vivo studies to compare the neurotoxic potencies of a number of quaternized nitrogen containing compounds with that of MPP^+. Since such charged, hydrophilic species will not readily pass the blood–brain barrier following systemic administration, intracerebral application is necessary for the in vivo assessment of their neurotoxic potency. Intrastriatal microdialysis is the method of choice for such studies because it allows the administration of a test compound to the target brain area via the dialysis probe and the simultaneous monitoring of its effects on various endogenous substances.[13] This technique has been employed previously to examine the effects of MPP^+ on DA efflux and lactate production[14,15] and was found to be an appropriate method to investigate the dopaminergic neurotoxicity of several MPP^+ analogues.[16,17] In addition, we have recently shown that intrastriatal administration of MPP^+ has similar effects in MPTP-sensitive species, such as the mouse and monkey, as in the rat.[18]

Chemistry. Most of the compounds examined in this study (Tables I and II) were prepared by literature methods or were obtained from commercial sources. The 1,2-dimethyl-4-phenylpyridinium species **7** was prepared via redox disproportionation of the corresponding 2,3-dihydropyridinium intermediate which in turn was obtained by treatment of MPP^+ with methylmagnesium iodide.

Intrastriatal Microdialysis. The use of microdialysis for studying the dopaminergic neurotoxicity of MPP^+ and analogues has been described recently.[15,17] Briefly, a U-shaped cannula with a cellulose dialysis membrane is implanted in the rat striatum. The next day (day 1) the cannula is perfused with Ringer solution and the perfusate is analyzed for DA and its metabolite by an on-line HPLC assay.[19] After the output of DA and metabolites has stabilized, the test compound is perfused for a fixed period and then the perfusion is continued with Ringer solution only. The acute effects of the test compound on the release of DA provides an index of its DA-releasing properties. As established with MPP^+,[15] toxic compounds also cause a prolonged release of DA which is a consequence of nerve-terminal damage. The sampling time of 20 min does not allow one to discriminate between the rapid, reversible DA-releasing effect and the delayed toxic effect. The acute, maximal drug-induced DA release (DA_{max}) is taken as a measure of the propensity of the test compound to be taken up into the nerve terminal via the DA carrier. The DA release after a challenge perfusion with 10 mM MPP^+ on day 2 (DA_{MPP^+}), 24 h after perfusion with the test compound, shows the extent to which the drug has caused persistent and presumably irreversible terminal lesion. After perfusions with nontoxic compounds, the MPP^+-induced DA release is comparable to that observed in controls animals while the effect of MPP^+ after perfusions with toxic compounds is reduced dramatically and often is not detectable. In addition, the basal levels of DA and metabolites one day after test drug perfusion can be related to drug-induced toxicity since the basal level of DA is below detection limits and the output of the metabolites DOPAC and HVA is persistently decreased to less than 20–40% of initial basal levels. In contrast, nontoxic compounds do not cause lasting effects on the basal DA levels and the metabolites' levels return to 70–100% of control levels 1 day later.

Effect on Lactate Formation. One of the proposed ultimate toxic events associated with the neurodegeneration caused by MPP^+ is inhibition of mitochondrial respiration.[20] One consequence of such inhibition is the

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compensatory increase in anaerobic glycolysis resulting in increased lactate production. Enhanced lactate production indeed occurs in vitro[21] and in vivo[15] following exposure to MPP⁺. Consequently, selected test compounds examined, again by in vivo microdialysis, to determine their capacity to increase striatal lactate formation in rat brain. Levels of lactate in brain dialysates have been measured by an on-line enzymatic assay in which newly formed NADH is estimated fluorometrically.[22] We have modified this method by measuring NADH electrochemically after HPLC separation in order to avoid fluorescence interference of the test compounds.

Results and Discussion

According to current theories, the MPP⁺-like dopaminergic neurotoxicity of a test compound as measured by microdialysis will be dependent on the following three discrete steps: (1) transport into the dopaminergic nerve terminal, (2) accumulation in the mitochondria, and (3) inhibition of mitochondrial respiration. In the present study, we have used a protocol in which the test compound is perfused as a 10 mM solution in Ringer solution for various time periods. Twenty-four hours later a challenge perfusion of 10 mM MPP⁺ is administered for 15 min and its effect on DA release is measured. The magnitude of this MPP⁺-induced DA release and the duration of the perfusion with the test compound 1 day earlier provide an estimate of the potency of the test compound relative to that of MPP⁺. Prolonged exposure of the striatum to a test compound which is nontoxic after short-lasting perfusion may increase intrastriatal concentrations of the test compound to such an extent that it exerts neurotoxic effects. The toxic potency of a compound therefore will be inversely related to the duration of the perfusion on day 1 required to cause a toxic response. We have used this rough guide as an index of the relative potencies of the test compounds and have ranked them in Table I as very toxic (15 min or less, group I), toxic (15–60 min, group II), moderately toxic (60–150 min, group III), weakly toxic (150–600 min, group IV), and nontoxic (>600 min, group V). Typical time–response curves for the effects of compounds from groups I, III, and V on extracellular DA and DOPAC levels are shown in Figure 1. Data on the acute increase in the release of DA on day 1 (DA_{max}) and on the effect of the MPP⁺ challenge on the DA release 24 h later (DA_{MPP+}) are summarized in Table I and discussed below. The effects of selected compounds on lactate formation are given in Table II as percentages of basal values.

Group I (1–3) consists of MPP⁺ (1) and the 2′-methyl and 4′-amino analogues **2** and **3**, which have comparable potencies to that of MPP⁺. The potent neurotoxicity of **2** and **3** are in agreement with recent in vitro data on the selective toxic effects of these pyridinium compounds on cultured dopaminergic neurons[11] as well as with the reported in vivo neurotoxicity of the parent tetrahydro analogues.[1,3f] The comparable effects of MPP⁺ and its 2′-methyl derivative on the release of DA and inhibition of mitochondrial respiration[9] in vitro are consistent with their similar in vivo neurotoxic profiles. Also, in vivo lactate production is enhanced by **2** to a similar extent as by MPP⁺ after a short-lasting perfusion (Table II).

Group II (4–13) consists of analogues which are only moderately toxic when perfused for 15 min but which are of comparable toxicity to MPP⁺ following a 60-min perfusion. In contrast to the toxic effects of these pyridinium

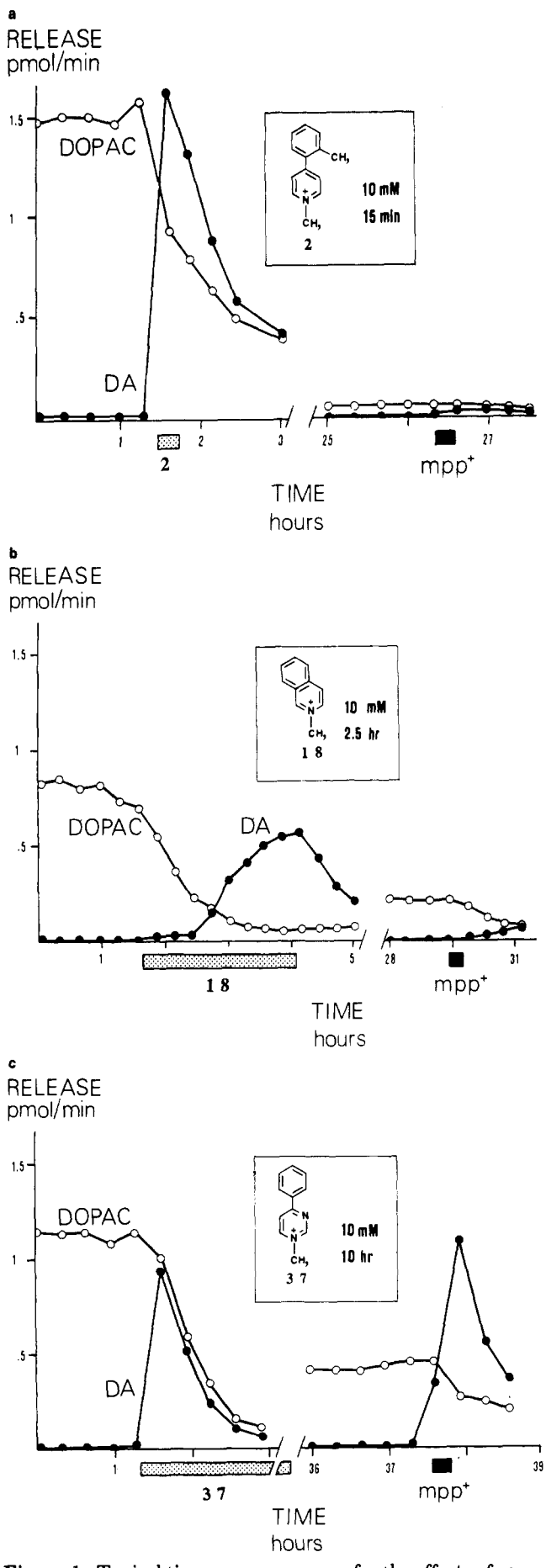

Figure 1. Typical time–response curves for the effects of compounds from groups I (a), III (b), and V (c) on extracellular DA and DOPAC following initial perfusion with the test compound and 24 h later following a challenge perfusion with MPP⁺.

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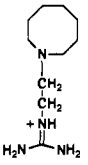
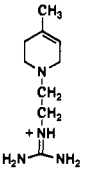
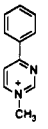
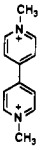
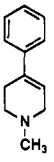
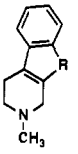
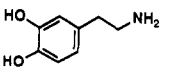
Table I. Effects of Various Pyridinium and Related Systems on Striatal Dopamine Levels in the Rat as Determined by Intracerebral Microdialysis

compound		DA release (% of basal × 10^{-3})	
no.	substituents	DA_{max}	DA_{MPP^+}
	Group I (15 min)		
1	$R_1 = R_2 = H$ (MPP^+)	21	0.15
2	$R_1 = H$, $R_2 = CH_3$	23	0.18
3	$R_1 = NH_2$, $R_2 = H$	20	0.56
	Group II (60 min)		
4	$R_1 = R_2 = H$, $R_3 = C_2H_5$	25	0.22
5	$R_1 = R_2 = H$, $R_3 = C_3H_7$	21	0.90
6	$R_1 = R_2$, $R_3 = CH_2$-cyclopropyl	23	1.10
7	$R_1 = H$, $R_2 = R_3 = CH_3$	14	0.59
8	$R_1 = CH_3$, $R_2 = H$, $R_3 = p$-$CH_3C_6H_4$	19	0.14
9	R_1 = cyclohexyl, $R_2 = H$, $R_3 = CH_3$	24	1.13
10	R_1 = 3-cyclohexenyl, $R_2 = H$, $R_3 = CH_3$	17	1.22
11	$R_1 = CH_2C_6H_5$, $R_2 = H$, $R_3 = CH_3$	19	0.39
12	$R_1 = N(CH_3)_2$, $R_2 = H$, $R_3 = CH_3$	17	0.86
13	$R_1 = H$, $R_2 = C_6H_5$, $R_3 = CH_3$	23	0.65
	Group III (150 min)		
14	$R_1 = C_6H_5$, $R_2 = H$, $R_3 = CH_2CCH$	25	0.04
15	$R_1 = C_6H_5$, $R_2 = H$, $R_3 = C_6H_5CH_2$	10	1.40
16	R_1 = *tert*-butyl, $R_2 = H$, $R_3 = CH_3$	8	2.90
17	$R_1 = H$, $R_2 = C_6H_5$, $R_3 = CH_3$	21	2.80
18	X = CH, Y = N^+CH_3	18	1.20
19	X = N^+CH_3, Y = CH	20	1.00
20		17	3.20
21	(*N*-Me-MPTP)	12	0.75
22	(debrisoquin)	8	1.05
	Group IV (300 min)		
23		5	0.27
24	R = H	6	1.70
25	R = CH_3	2	2.05
26		3	2.05

Table I (Continued)

	compound		DA release (% of basal × 10^{-3})	
	no.	substituents	DA_{max}	DA_{MPP^+}
	27	(guanethidine)	3	2.70
	28	(guanacline)	4	2.90
		Group V (600 min)		
	29	R_1 = H, R_2 = CH_3	0.58	18
	30	R_1 = CH_2OH, R_2 = CH_3	0.20	24
	31	R_1 = $CONH_2$, R_2 = CH_3	0.25	25
	32	R_1 = $COOCH_3$, R_2 = CH_3	0.15	18
	33	R_1 = p-$C_6H_4CH_2OH$, R_2 = CH_3	0.22	11
	34	R_1 = H, R_2 = $(CH_2)_{15}CH_3$	0.18	24
	35	R_1 = CH_3	0.25	31
	36	R_1 = $CH_2C_6H_5$	1.70	17
	37		22	21
	38	(paraquat)	0.18	18
	39	X = CH, Y = N (isoquinoline)	0.40	18
	40	X = N, Y = CH (quinoline)	0.50	23
	41	R = H	1.20	22
	42	R = CH_3	1.22	17
	43	R = H	1.51	20
	44	R = CH_3	1.30	28
	45	(MPTP)	2.40	16
	46	R = CH_2	1.31	19
	47	R = NH	1.80	19
	48	(dopamine)	not determined	17

[a] After obtaining a stable basal striatal DA release (4.6 ± 0.3 fmol/min, n = 123), test compounds were perfused intrastriatally as 1 mM solutions in Ringer solution for the indicated time. Increases in DA release are expressed as percentages of the basal release. [b] DA_{max}: Maximal DA released induced by perfusion with the test compound. [c] DA_{MPP^+}: Maximal DA release induced by perfusion with 10 mM MPP^+ (15 min) 1 day after perfusion with the test compound.

species, the majority of the corresponding tetrahydro derivatives lack MPTP-type neurotoxic effects in the mouse model,[1,2] presumably because of their poor MAO substrate properties. Consistent with this view, the tetrahydro derivative of the cyclohexyl compound **9**, a good MAO-B substrate, is neurotoxic in the mouse[23] while the tetrahydro

Table II. Effects of Various Pyridinium and Related Systems on Striatal Lactate Levels in the Rat as Determined by Intracerebral Microdialysis

compd	no.	min	lactate efflux (% of basal)[a]
MPP^+	1	1	210
		15	280
2′-CH_3MPP^+	2	1	155
2-PMP^+	7	1	200
4′-CH_3MPP^+	8	1	180
		15	230
3-PMP^+	13	15	240
4-*t*-BMP^+	16	15	106
2-PMP^+	17	15	160
N-MIQ^+	19	15	190
		150	200
Pym^+	37	15	102
THIQ	43	150	98

[a] Basal lactate efflux was measured in each rat before starting the drug perfusion. Changes were calculated as percentages of the corresponding basal level. Average basal lactate efflux was 689 ± 103 pmol/min (n = 23). Each experiment was performed at least in duplicate.

analogue of 2-phenyl isomer **13** of MPP^+, which has no measurable substrate activity, is devoid of toxic effects in the mouse.[2a] Likewise the 2-methyl and 4′-methyl tetrahydro derivatives corresponding to **7** and **8** are not neurotoxic in the mouse even though **7** and **8** were found to be potent inhibitors of mitochondrial respiration in vitro[9,24] and to increase lactate formation in vivo in response to a short-lasting intrastriatal perfusion (Table II).

An exception to these generalizations is the tetrahydro derivative of the 1-methyl-4-benzylpyridinium compound (**11**) which is an excellent MAO-B substrate but is not neurotoxic in the mouse[25] even at 10 times the fully toxic dose of MPTP.[1b] Since the expected pyridinium metabolite **11** has been shown in vitro to inhibit mitochondrial respiration[9,10] and is a potent DA releaser in our dialysis model, its in vivo MPP^+-type neurotoxicity is not surprising. A recent report[11] found **11** to be toxic to cultured rat embryonic cells but the toxic effects were not selective for dopaminergic neurons. In vitro studies have confirmed that **11** is formed from the tetrahydro compound in a reaction catalyzed by MAO-B.[27] Additional studies on the in vivo metabolic fate of the tetrahydro compound will be necessary to clarify the apparently conflicting outcomes. The toxic properties of the hydrophilic 4-dimethylamino derivative **12** also are noteworthy in that this system is structurally quite distinct from all other reported MPP^+-type toxins. The electronic properties of the dimethylamino group with results in enhanced delocalization of the positive charge appears to be associated with increased toxic potency. This analysis also may explain the potent toxicity of 4′-amino analogue **3** relative to that of the corresponding 4′-methyl analogue **8**.

This group also contains several *N*-alkyl analogues of MPP^+ with appreciable toxicity. It is of interest to note that the *N*-ethyl and the *N*-propyl analogues **4** and **5** are of comparable potency in our system and in inhibiting mitochondrial respiration[9] whereas only the *N*-ethyl analogue **4** displayed selective dopaminergic toxicity in cell cultures.[11] This difference in activities might be explained by the poorer in vitro uptake characteristics of the *N*-propyl compound.[9] This interpretation, however, is not consistent with our in vivo DA release data which show that both compounds induce essentially the same acute increase in DA release.

Group III (14–22) is composed of quaternary species which require perfusions of more than 60 min to display an MPP^+-type neurotoxic effect. The neurotoxicity of *N*-methyl derivative **21** of MPTP and the lack of toxic effects of MPTP (**45**) itself support the view that a permanent charge on the heterocyclic nitrogen atom is required to elicit MPP^+-like effects, perhaps because the fixed charge facilitates accumulation in the mitochondria.[10,26] The dopaminergic toxicity of the guanidine derivative debrisoquin (**22**) (and of guanethidine (**27**) and guanacline (**28**), group IV) may be related to the fact the guanidines, which are known to be electron-transport inhibitors,[27] are permanently charged at physiological pH. Recent in vitro studies using dopaminergic neurons in culture have shown toxic effects of guanidines although toxicity was nonselective.[11]

2-Phenyl isomer **17** of MPP^+ is a somewhat weaker toxin than 3-phenyl isomer **13**, a result which parallels the weaker potency of **17** in inhibiting NADH oxidase activity in inner mitochondrial membrane preparations[24] and in increasing in vivo lactate formation (Table II).

N-Methylisoquinolinium species **18** shows toxic effects after a 60-min perfusion and increased lactate formation after a 15-min perfusion (Table II). These results are of particular interest because tetrahydroisoquinolines (and tetrahydro-β-carbolines, see below) have been detected in rat and human brain[29] and have been implicated in the etiology of Parkinson's Disease.[30] It has for example been reported that tetrahydroisoquinoline (**43**) produces a parkinsonian syndrome in marmosets.[31] It is unlikely, however, that tetrahydro compound **43** or its *N*-methyl analogue **44** are MPP^+-type neurotoxins since they are devoid of any toxic effects after long-lasting intrastriatal perfusions (see below, group V). In addition, high doses of **43** and **44** did not show dopaminergic toxicity in mice[2d] and in marmosets,[2b] respectively. For the in vivo formation of the more likely neurotoxin methylisoquinolinium (**18**), two possible pathways have been proposed: tetrahydroisoquinoline (**43**) can be N-methylated[32a] to *N*-methyltetrahydroisoquinoline (**44**) which, as a substrate for MAO,[16b,32b] can be metabolized to isoquinolinium species **18**. Alternatively, **43**, also an MAO substrate,[16b] could be oxidized in vivo to isoquinoline (**39**) followed by N-methylation to **18** via brain methyl transferases which have been shown to methylate similar heterocyclic systems. *N*-Methylisoquinolinium derivative **18** was also found to be a neurotoxin in cultured neurons in vitro, however, again without appreciable selectivity for dopaminergic neurons.[11] Although isomeric *N*-methylquinolinium

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species **19** has a comparable neurotoxic potency in our model as **18**, it is not likely to be involved in the etiology of Parkinson's Disease since the corresponding tetrahydro derivatives **41** and **42** are not substrates for MAO-B.[16b]

Tetrahydroindenopyridine **46** of indenopyridinium compound **20** was synthesized as a carbon analogue of tetrahydro-β-carboline **47** to explore electronic effects on the requirements for MAO-B substrates.[33] Both tetrahydro compounds are poor MAO-B substrates, having only 0.4% and 0.8%, respectively, of the activity of MPTP. The moderate to weak toxicities of indenopyridinium **20** and β-carbolinium **26** together with the poor substrate properties of the corresponding tetrahydro compounds are consistent with the lack of in vivo toxicity of the tetrahydro derivatives.[2a,33,34] Indenopyridinium compound **20** was found to be taken up by striatal synaptosomes to a similar extent as cyclohexylpyridinium compound **9**,[17] in agreement with their comparable toxicities.

Finally, the activity of *tert*-butyl compound **16** is unexpected since in vitro data show that it is a poor substrate for the DA carrier[9] and a poor inhibitor of mitochondrial respiration.[9,24] In addition, it was found that its tetrahydro analogue, although being a rather good substrate for MAO-B, was devoid of any toxic effects in the mouse.[1] Nevertheless, intrastriatal perfusion caused not only a pronounced DA release, indicating uptake into the dopaminergic nerve terminal, but also irreversible effects on the DA release when perfused for more than 1 h.

Group IV (23–28) is composed of compounds which are only toxic after long-lasting perfusions, showing 0.2–1% of the potency of MPP+. Among these are two guanidine derivatives guanethidine (**27**) and guanacline (**28**), compounds which have been shown to produce sympathetic neuronal destruction in rats and monkeys.[35] As already mentioned, several guanidines, including guanethidine, were found to be moderately potent toxins in cultured cells without selectivity for dopaminergic cells.[11]

The *N*-methyl derivative of the fully saturated analogue of MPTP, *N*,*N*-dimethylpiperidine **26**, is estimated to be about 10 times less potent than the *N*-methyl quaternary derivative (**21**) of MPTP itself. This indicates that unsaturation of the nitrogen-containing ring increases the potency, reflecting improved uptake or inhibitory properties.

The low potency of another N-substituted analogues of MPP+, *N*-hydroxyethyl derivative **23**, clearly illustrates the role of steric and lipophilic factors for MPP+-type toxicity. The increased polarity of the hydroxyethyl group in **23** and the greater bulk and lipophilicity of the *N*-benzyl moiety in **15** in comparison with the *N*-ethyl and *N*-propyl compounds **4** and **5**, are apparently unfavorable for neurotoxicity.

Finally, two β-carbolinium species, **24** and **25**, were found to possess weak MPP+-like toxicity. As in the case of the tetrahydroisoquinoline derivatives (see above), tetrahydro-β-carbolines have been detected in the brain[36] and have been suggested to be potential endogenous MPTP-like toxins.[34] Although *N*-methyltetrahydro-β-carboline (**47**) was not toxic after systemic administration,[2a,34b] it was recently found to be a weak MAO-B substrate.[16b,33] The activity of these β-carbolinium species is in agreement with the fact that some were found to be weak substrates for the DA uptake carrier[17] and good inhibitors of mitochondrial respiration in vitro,[10] although *N*-methyl-β-carbolinium acted as an uncoupler rather than as an inhibitor.

Group V (29–48) consists of compounds which did not display toxic effects even after long-lasting perfusions. With the exception of pyrimidinium analogue **37** (see below), perfusions with these compounds for 600 min resulted only in a small or moderate release of DA and in a full DA release response (17 000–25 000%) following the MPP+ challenge. This group includes all species bearing no positive charge, thus demonstrating that only permanently charged compounds have MPP+-like properties when administered intrastriatally in rats. The lack of toxic effects of a 10-h perfusion of MPTP (**45**) argues that under microdialysis conditions MPTP is not converted to toxic levels of MPP+. Likewise, the other neutral compounds, 2-methyltetrahydro-β-carboline (**47**), the related tetrahydroindenopyridine **46**, tetrahydroisoquinolines **43** and **44**, tetrahydroquinolines **41** and **42**, isoquinoline (**39**), and quinoline (**40**) are inactive in this assay. As would be predicted for these nontoxic compounds, tetrahydroisoquinoline (**43**) failed to increase lactate production in vivo (Table II).

The presence of a pyridinium moiety is not sufficient to insure neurotoxic effects. Replacement of the lipophilic phenyl group present in MPP+ with a polar functionality such as (hydroxymethyl)phenyl (**33**), hydroxymethyl (**30**), methoxycarbonyl (**32**), or aminocarbonyl (**31**) leads to inactive compounds. Furthermore, it appears that the absence of a lipophilic group is also detrimental since the monosubstituted *N*-methylpyridinium species **28** is devoid of activity. Although pyridinium species with long alkyl chains as N-substituents are extremely potent inhibitors of mitochondrial respiration, the lipophilic *N*-cetylpyridinium compound **34** also was inactive in terms of its effects on DA release and in terms of lactate production. The inactivity of paraquat (**38**) may be due to the presence of two positive charges which may prevent transport into the nerve terminals.

The observations that pyrimidinium species **37** is as effective as MPP+ in causing DA release but is devoid of MPP+-like neurotoxic effects is very interesting since it obviously can enter the dopaminergic nerve terminal and, as a permanently charged species, it is expected to have access to the mitochondria. However, although **37** is a potent inhibitor of complex I in inverted mitochondria, it does not inhibit electron transport in intact mitochondria.[10] Its lack of effect on lactate production (Table II) is in agreement with this finding. Therefore its inactivity might be related to a poor accumulation into the mitochondria, although the reason for this is not understood.

Conclusions

A comparison of the compounds examined in this series suggests that electronic, steric, and lipophilic factors all contribute to the biological activity of this system. Only compounds bearing a permanent positive charge are neurotoxic in this assay. Since a variety of pyridinium and related systems are toxic, it would appear that the uptake and accumulation of quaternary species into mitochondria are not dependent on a specific transport mechanism. This agrees with the recent reports that the mitochondrial uptake MPP+ occurs by a passive, Nernstian transport mechanism in response to the transmembrane electrochemical potential gradient.[10,26] Since in addition the

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structural requirements for inhibitors of mitochondrial respiration do not appear to be very strict,[10] it is not surprising that we find many charged compounds to be neurotoxic. It is therefore reasonable to speculate that the rate-limiting step in the expression of a pyridinium-type neurotoxicity is the uptake into the nerve terminal. Consequently, long-term exposure to quaternary pyridinium compounds which are poor substrates for the DA carrier, such as *N*-propyl analogue **5**, may still induce neuronal lesions as shown by our results with long-lasting perfusions.

It appears that toxicity is enhanced by delocalization of the pyridinium charge as 4′-amino derivative **3** is considerably more toxic than 4′-methyl MPP^+ analogue **8**, and 4-dimethylamino derivative **12** is more toxic than 4-*tert*-butyl analogue **16**. Somewhat puzzling in this regard is the high activity of 2′-methyl analogue **2** since this substitution pattern leads to the loss of coplanarity of the phenyl and pyridinium rings and hence loss of the extent of delocalization of the pyridinium charge. However, since 4-cyclohexyl analogue **9** is a potent toxin, such delocalization of charge, while favorable, is not an essential feature for activity. The moderate toxicity of *N*-methyl analogue **21** of MPTP may likewise be due to the absence of a delocalized organic cationic moiety but also may be a consequence of steric effects.

Another important feature influencing activity is the hydrophilicity of the molecule. In general, the introduction of a polar group into the already hydrophilic pyridinium species abolishes all toxicity. This is most strikingly demonstrated by the decrease in activity of the hydroxyl-containing compounds **23** (*N*-hydroxyethyl) and **33** (4′-hydroxymethyl) compared with the corresponding *N*-ethyl and *N*-methyl derivatives **4** and **8**, respectively. The relatively potent toxicity of the amino-containing pyridinium compounds **3** and **12** may reflect the enhanced delocalization of the positive charge in these derivatives. These results, which reflect a fairly limited range of lipophilicities, do not provide much insight into the possible toxicity-enhancing potential of increased lipophilicity. As discussed below, the possibility of exploring the influence of increased lipophilicity on toxicity is somewhat hampered because of the concomitant changes in the steric features of the molecules.

The steric bulk around the pyridinium nitrogen atom greatly affects the activity of this series. For example, replacement of the N-substituent of MPP^+ with a larger group such as ethyl (**4**) or propyl (**5**), which at the same time increases the lipophilic character of the molecules, leads to a decrease in toxicity. Similarly, 2-methyl analogue **7** of MPP^+ is 1 order of magnitude less toxic then MPP^+ while the toxicity of 3-phenyl isomer **17** is further decreased. It is likely that both steric and electronic factors play a role in the activity of the bicyclic and tricyclic compounds.

A review of these results suggests that all toxic compounds are also highly effective DA releasers. One possible interpretation of this relationship is that the initial release of DA is a consequence of active transport of the pyridinium compounds into nerve terminals following which irreversible damage is caused as a consequence of the inhibition of mitochondrial respiration. Alternatively, it may be argued that DA, a reported neurotoxin released as a consequence of the perfusion, is itself responsible for the nerve-terminal damage. In our hands, however, perfusion of the rat striatum with 10 mM DA for 600 min was without effect on the release of DA from the nerve terminals by MPP^+ following a 24-h washout period. Furthermore, results obtained with pyrimidinium analogue **37** of MPP^+ provides an example, although the only such example thus far, of a compound which has good DA-releasing properties but causes no irreversible effects on the dopaminergic system. This result is also in agreement with earlier reports that DA is not required for MPTP toxicity.[2a] The results of our study of 47 MPP^+ and MPTP derivatives and related compounds clearly shows that MPP^+ is a unique neurotoxin. None of the modifications of the MPP^+ studied lead to a more potent compound. On the other hand, the fact that a variety of compounds exhibit neurotoxic effects in vivo indicates that a search for endogenous or environmental MPTP-like toxins should include permanently positively charged species or compounds which can be transformed into charged derivatives in vivo but which are not necessarily closely related to MPTP.

Experimental Section

MPP^+ iodide (**1**·I) debrisoquin (**22**), guanethidine (**27**), *N*-cetylpyridinium iodide (**34**·I), paraquat (**38**), isoquinoline (**39**), quinoline (**40**), tetrahydroquinoline (**41**), tetrahydroisoquinoline (**43**), MPTP hydrochloride (**45**·HCl), and dopamine hydrochloride (**48**·HCl) were obtained from commercial sources. The iodide salts of 1-ethyl-4-phenylpyridinium iodide (**4**·I),[37] 1-propyl-4-phenylpyridinium iodide (**5**·I),[38] 1-methyl-4-cyclohexylpyridinium iodide (**9**·I),[27] 4-benzyl-1-methylpyridinium iodide (**11**·I),[39] 1-benzyl-4-phenylpyridinium chloride (**15**·Cl),[37] 1-methyl-4-*tert*-butylpyridinium iodide (**16**·I),[40] 2-methylisoquinolinium iodide (**18**·I),[41] 1-methylquinolinium iodide (**19**·I),[42] 2-methyl-9*H*-indeno[2,1-*c*]pyridinium chloride (**20**·Cl),[43] methiodide **21** of MPTP,[44] 1-(2-hydroxyethyl)-4-phenylpyridinium bromide (**23**·Br),[45] 2-methyl-β-carbolinium iodide (**24**·I),[46] 1,2-dimethyl-β-carbolinium iodide (**25**·I),[47] 1,1-dimethyl-4-phenylpiperidinium iodide (**26**·I),[48] 1-methylpyridinium iodide (**29**·I),[40] 4-(aminocarbonyl)-1-methylpyridinium iodide (**31**·I),[49] 4-(methoxycarbonyl)-1-methylpyridinium iodide (**32**·I),[50] 3-(aminocarbonyl)-1-methylpyridinium iodide (**35**·I),[51] 3-(aminocarbonyl)-1-benzylpyridinium bromide (**36**·Br),[52] 1-methyl-4-phenylpyrimidinium iodide (**37**·I),[53] *N*-methyltetrahydroquinoline (**42**),[43] *N*-methyltetrahydroisoquinoline (**44**),[41] 1,2,3,4-tetrahydro-2-methyl-9*H*-indeno[2,1-*c*]-pyridine hydrochloride (**46**·Cl),[33] and 2-methyl-1,2,3,4-tetrahydro-β-carboline hydrochloride (**47**·Cl)[46] were prepared according to the cited literature. 4-(4-Aminophenyl)-1-methylpyridinium iodide (**3**·I, Dr. J. Johannenssen, NIH), guanacline (**28**, Dr. M. Palmatier, NIH), and 4-(3-cyclohexenyl)pyridine (Reilly Tar and Chemical Co.) were obtained as gifts. All compounds gave ac-

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ceptable melting points and ^1H NMR spectra. Synthetic reactions were carried out under a nitrogen atmosphere. All chemicals were reagent or HPLC grade. Proton NMR spectra were recorded on a General Electric 500-MHz or Bruker WP 270-MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to Me_4Si as an internal standard and spin multiplicities are given as s (singlet), d (doublet), t (triplet), or m (multiplet). Fast atom bombardment (FAB) mass spectral analyses were performed on a VG 7070E-HF mass spectrometer using a glycerol matrix. Unless otherwise specified, biological studies were run in 50 mM, pH 7.4 phosphate buffer. Enzyme reaction rates were monitored on a Beckman Model 50 spectrophotometer. Quantitative and qualitative HPLC analyses were performed on a Beckman 114M chromatograph employing an HP Model 1040A diode-array detector. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by the Atlantic Microlab, Inc., Norcross, GA.

1-Methyl-4-(2-methylphenyl)pyridinium Iodide (2′-Methyl-MPP+ Iodide, 2·I). A solution of 2-bromotoluene (1.7 g, 10 mmol) in diethyl ether (20 mL) was added dropwise to a stirred solution of *n*-butyllithium in hexane (7 mL of a 1.6 M solution, 11 mmol) and diethyl ether (25 mL) cooled with a dry ice/acetone bath. Thirty minutes after the addition was completed a solution of 1-methyl-4-piperidone (1.1 g, 10 mmol) in diethyl ether (20 mL) was added dropwise. The ice bath was removed when the addition was complete and the solution was stirred for 1 h at room temperature. The reaction mixture then was poured onto ice and the resulting mixture was acidified with 10% HCl and then washed with diethyl ether. The aqueous extract was basified with 50% NaOH and extracted three times with methylene chloride, and the combined extracts were dried (Na_2SO_4), filtered, and concentrated to give the crude alcohol 4-hydroxy-1-methyl-4-(2-methylphenyl)piperidine as a viscous yellow oil. A 1:3 mixture of HCl/acetate acid (30 mL) was added to the crude alcohol and the resulting solution was stirred under reflux for 10 h. After cooling, ice was added and the solution was basified with 50% NaOH and then extracted three times with methylene chloride. The extracts were combined, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography (silica gel, 95:5 methylene chloride/methanol) and the purified product was converted to its HCl salt by bubbling HCl gas into an acetone solution of the compound. The white solid that precipitated was filtered, washed with acetone/ethyl ether and recrystallized from diisopropyl ether/ethanol to yield 0.9 g (40%) of 1-methyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine: mp 224–6 °C; ^1H NMR (free base in $CDCl_3$) δ 7.15 ppm (m, 4 H, C_6H_4), 5.54 (m, 1 H, C-5), 3.10 (m, 2 H, C-6), 2.65 (t, 2 H, C-2), 2,42 (s, 5 H, NCH_3 and C-3), 2.30 (s, 3 H, $C_6H_4CH_3$); ^1H NMR of HCl salt (D_2O) δ 7.20 (m, 4 H, C_6H_4), 5.58 (s, 1 H, C-5), 4.08 (d, 1 H, C-6), 3.57 (m, 1 H, C-2), 3.47 (d, 1 H, C-6), 3.17 (m, 2 H, C-3, C-2), 2.91 (s, 3 H, NCH_3), 2.48 (m, 1 H, C-3), 2.34 (s, 3 H, $C_6H_4CH_3$). Anal. ($C_{13}H_{17}N$·HCl).

The free base obtained from the above tetrahydropyridine hydrochloride (224 mg, 1 mmol) was heated under reflux in 20 mL of *o*-xylene in the presence of 200 mg of 10% Pd/C for 12 h. Thin-layer chromatography (93:7 methylene chloride/methanol) indicated that a compound with UV absorption, iodine reaction, and chromatographic mobility similar to 4-phenylpyridine has been formed. Apparently, aromatization and demethylation had occurred, giving 4-(2-methylphenyl)pyridine as the major product. The catalyst was filtered off and the filtrate was extracted three times with 10% HCl. The combined aqueous extracts were basified with NaOH and extracted three times with methylene chloride. The combined organic extracts were dried with sodium sulfate, filtered, and concentrated to an oil. The compound which comigrated with 4-phenylpyridine on TLC was purified by column chromatography (silica gel, 98:2 methylene chloride/methanol). The oily residue obtained after evaporation of the solvent was dissolved in 15 mL of acetone, and 1 mmol of iodomethane was added. The solution was stirred overnight and the yellow crystals (needles) which formed were filtered, washed with cold acetone, and dried to yield 95 mg (30%) of the product: mp 170–2 °C; EIMS *m/e* 169 ($M^{\cdot+} - CH_3$); ^1H NMR ($CDCl_3$) δ 9.20 (d, 2 H, C-2, C-6), 7.91 (d, 2 H, C-3, C-5), 7.30 (m, 4 H, C_6H_4), 4.67 (s, 3 H, NCH_3), 2.30 (s, 3 H, $C_6H_4CH_3$). Anal. ($C_{13}H_{14}IN$) C, H, I, N.

1-(Cyclopropylmethyl)-4-phenylpyridinium Bromide (6·Br). A solution of cyclopropylmethyl bromide (2.0 g, 14.8 mmol) and 4-phenylpyridine (0.77 g, 4.9 mmol) in 8 mL of dry acetone was stirred at room temperature for 18 h. Recrystallization of the resulting solid from methanol/anhydrous diethyl ether gave 0.26 g (18%) of the desired product: mp 238–239 °C; ^1H NMR ($CDCl_3$) δ 9.60 ppm (d, 2 H, C-2, C-6), 8.25 (d, 2 H, C-3, C-5), 7.5–7.8 (m, 5 H, C_6H_5), 4.90 (d, 2 H, $C_3H_5CH_2$), 1.6 (m, 1 H, CH_2CH), 0.8–0.9 (m, 4 H, CH_2CH_2). Anal. ($C_{15}H_{16}BrN$) C, H, N.

1,2-Dimethyl-4-pyridinium Perchlorate (7·ClO_4).[55] To a suspension of MPP+ iodide (10.0 g, 34 mmol) in 700 mL of dry diethyl ether was added a 2.9 M solution of methylmagnesium iodide (60 mL, 174 mmol) at room temperature with stirring over a period of 20 min. After heating under reflux for 2 h, the excess Grignard reagent was decomposed by the careful addition of 1 M aqueous HCl (8 mL). The next day the ether layer was removed and the gummy aqueous layer was saturated with sodium chloride and extracted into dichloromethane (10 × 200 mL). HPLC analysis [Altex Ultrasil-cx 4.6 × 25 mm column, acetonitrile (15%) in 0.1 M acetic acid/0.05 M triethyl ammonium chloride made to pH 2.3 with formic acid] of the organic-soluble material confirmed the presence of the desired pyridinium product 7 (t_R = 8 min) together with the corresponding 2-methyl- and 6-methyl-1,2,3,6-tetrahydropyridines (t_R of both 4.0 min) formed in the redox disproportionation reaction. The residue obtained from the above extracts in 200 mL of water made to pH 9 with 1 N sodium hydroxide was extracted with diethyl ether (2 × 200 mL) to remove the tetrahydropyridines. The aqueous layer was made acidic with concentrated hydrochloride acid and then extracted with dichloromethane (4 × 200 mL). After drying over magnesium sulfate and removal the solvent, the crude pyridinium product (3.3 g, 62%) was obtained as a yellow solid. Crystallization from acetonitrile yielded 2.0 g (38%) of the iodide as a hydrate: mp 187–9 °C; ^1H NMR ($CDCl_3$) δ 9.50 ppm (d, 1 H, C-6), 8.10 (m, 2 H, C-3 and C-5), 7.60–7.75 (m, C_6H_5), 4.5 (s, 3 H, NCH_3), and 3.0 (s, 3 H, CCH_3); UV (CH_3OH λ_{max} 284 nm, ϵ 17 500. Anal. $C_{13}H_{14}IN·1.7H_2O$. The iodide salt (3.70 mg, 1.2 mmol) in 25 mL of water was treated with aqueous silver perchlorate (2.81 mg, 1.36 mmol in 10 mL water). The resulting precipitate was filtered and washed, and the combined aqueous solutions were lyophilized to yield a white solid which, after recrystallization from acetone, gave the pure perchlorate salt: mp 160–1 °C. Anal. $C_{13}H_{14}ClNO_4$.

1-Methyl-4-(4-methylphenyl)pyridinium Iodide (8·I). Following the procedure described above for the preparation of 2′-MPP+ iodide (2·I), the reaction of (4-methylphenyl)magnesium bromide with 1-methyl-4-piperidone gave the corresponding alcohol, which underwent dehydration to yield the hydrochloride salt of 1-methyl-4-(4-methylphenyl)-1,2,3,6-tetrahydropyridine in 37% overall yield: mp 206 °C; ^1H NMR ($CDCl_3$) δ 7.30 (d, 2 H, C-2, C-6 of C_6H_4), 6.95 (d, 2 H C-3, C-5 of C_6H_4), 5.98 (s, 1 H, C-5), 4.02 (d, 1 H, C-6), 3.82 (m, 1 H, C-2), 3.51 (d, 1 H, C-6), 3.20 (m, 2 H, C-2, C-3), 2.91 (s, 3 H, NCH_3), 2.74 (d, 1 H, C-3), 2.40 (s, 3 H, $C_6H_4CH_3$). Anal. ($C_{13}H_{17}N$·HCl) C, H, N.

The free base obtained from the above tetrahydropyridine compound when heated under reflux in xylene in the presence of 10% Pd/C for 2 h underwent dehydrogenation and demethylation. The crude 4-(4-methylphenyl)pyridine obtained was treated with methyl iodide and the yellow needlelike crystals which separated were filtered, washed with acetone, and dried to yield 35% of the product: mp 165–8 °C; ^1H NMR (D_2O) δ 8.70 (d, 2 H, C-3, C-6), 8.25 (d, 2 H, C-3, C-5), 7.84 (d, 2 H, C-2, C-6 of C_6H_4), 7.47 (d, 2 H, C-3, C-5 of C_6H_4), 4.33 (s, 3 H, NCH_3), 2.44 (s, 3 H, $PhCH_3$). Anal. ($C_{13}H_{14}IN$) C, H, I, N.

1-Methyl-4-(3-cyclohexenyl)pyridinium Iodide (10·I). A mixture of freshly distilled 4-(3-cyclohexenyl)pyridine (16.3 g, 102 mmol, 16 mL) and methyl iodide (57 g, 402 mmol, 25 mL) in methanol (15 mL) was stirred under reflux for 3.5 h and then at room temperature overnight. The solvent was evaporated to yield a golden oil which crystallized upon standing. The crystals were

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filtered, washed with methanol/ether (1:4), and dried in vacuo to give the pure product: mp 119–121.5 °C; ^{1}H NMR ($CDCl_3$) δ 9.22 ppm (d, 2 H, C-2, C-6), 7.88 nd, 2 H, C-3, C-5), 5.76 (m, 2 H, C*H*=C*H*), 4.64 (s, 3 H, NCH_3), 3.1 (m, 1 H, C-1 of C_6H_9), 1.7–2.2 (m, 6 H, CH_2's of C_6H_9). Anal. ($C_{12}H_{16}NI$) C, H, N.

4-(Dimethylamino)-1-methylpyridinium Iodide (12·I). A solution of 4-(dimethylamino)pyridine (1.22 g, 10 mmol) and methyl iodide (1.42 g, 10 mmol) in acetone was stirred overnight at room temperature to give the pure product in 90% yield: mp 240–1; ^{1}H NMR δ 8.35 ppm (d, 2 H, C-2, C-6), 6.9 (d, 2 H, C-3, C-5), 4.12 (s, 3 H, NCH_3), 3.24 [s, 6 H, $N(CH_3)_2$]. Anal. ($C_7H_{10}N_2$) C, H, N.

1-Methyl-3-phenylpyridinium Iodide (13·I). This compound was prepared in 80% yield by following the procedure described above for 10·I: mp 152–3 °C; ^{1}H NMR ($CDCl_3$) δ 9.40 ppm (d, 1 H, C-2), 9.20 (d, 1 H, C-6), 8.60 (t, 1 H, C-5), 8.18 (t, 1 H, C-4), 7.6–8.0 (m, 5 H, C_6H_5), 4.20 (s, 3 H, CH_3). Anal. ($C_{12}H_{12}IN$), C, H, N.

4-Phenyl-1-(2-propynyl)pyridinium Bromide (14·Br). A mixture of 1-bromo-2-propyne (2.8 g, 25 mmol) and 4-phenylpyridine (1.0 g, 6.4 mmol) in 10 mL of dry acetone was stirred at room temperature for 24 h. Recrystallization of the resulting precipitate from absolute ethanol afforded 1.6 g (91%) of the desired product: mp 194–5 °C (lit.[52] mp 206–8 °C, lit.[53] mp 252–3 °C); ^{1}H NMR (DMSO-d_6) δ 9.25 ppm (m, 2 H, C-2, C-6), 8.6 (m, 2 H, C-3, C-5), 7.7–8.2 (m, 5 H, C_6H_5), 5.75 (d, 2 H, CH_2), 4.1 (d, 1 H, CCH). Anal. ($C_{14}H_{12}BrN$) C, H, N.

1-Methyl-2-phenylpyridinium Iodide (17·I). This compound was prepared in 75% yield following the procedure described above for 10·I: mp 141–2 °C; ^{1}H NMR ($CDCl_3$) δ 9.60 ppm (d, 1 H, C-6), 8.61 (t, 1 H, C-5), 8.22 (t, 1 H, C-4), 7.96 (d, 1 H, C-3), 7.6 (m, 5 H, C_6H_5), 4.40 (s, 3 H, CH_3). Anal. ($C_{12}H_{12}IN$) C, H, N.

4-(Hydroxymethyl)-1-methylpyridinium Iodide (30·I). This compound was prepared in 62% yield by following the procedure described above for 10·I: mp 101–3 °C; ^{1}H NMR (CD_3CN) δ 8.50 (d, 2 H, C-2, C-6), 7.86 (d, 2 H, C-3, C-5), 4.8 (d, 2 H, CH_2), 4.2 (s, 3 H, CH_3). Anal. ($C_7H_{10}NO$) C, H, N.

Intracerebral Microdialysis of DA and Metabolites. Microdialysis was performed with a fully automated system essentially as described elsewhere.[13,15] Male Sprague–Dawley rats (220–240 g, Bantin-Kingman, Fremont, CA) were anesthetized with chloral hydrate (400 mg/kg ip) and placed in a stereotaxic frame (Kopf). The skull was exposed and small holes were drilled to allow implantation of the bilateral U-shaped cannula with a cellulose dialysis membrane (MW cutoff 10000 Da, Cordis Dow, Brussels, Belgium) in both striata. Coordinates were *A* 7.5, *V* 6.0, and *L* 3.0.[54] The cannula was fixed to the skull with dental cement and an anchoring screw and the rat was allowed to recover. All experiments were performed one day after surgery. The inlet and outlet of the cannula were connected to a perfusion pump (Microperpex, LKB) and a HPLC valve, respectively, with flexible polyethylene tubes (inner diameter 0.28 mm). The striatum was perfused with a Ringer solution at a flow rate of 4.2 μL/min. The injector valve, equipped with an electric actuator and digital valve sequencer (Valco, Houston, Texas) is held in the load position for 20 min, in which the sample loop (50 μL) is filled with dialysate and then automatically switched to inject for 15 s. This procedure is repeated every 20 min, the time needed to record a complete chromatogram. DA, DOPAC, 5-HIAA, and HVA were estimated by reversed-phase HPLC and electrochemical detection, using a Beckmann Ultrasphere 5C18 column (250 × 4.6 mm), an Altex 110A pump, which delivered the mobile phase (2 mM heptanesulfonic acid and 10% methanol in 0.05 M sodium acetate buffer pH 3.6) at a flow rate of 1.0 mL/min, and a carbon paste BAS LC-3 Amperometric detector (BAS) set at 650 mV.

Each test compound was perfused on day 1 intrastriatally via the dialysis membrane as a 10 mM solution in Ringer Solution after the basal output of DA and metabolites had stabilized. One day after the end of the drug perfusion, the striatum was perfused again on day 2 with Ringer Solution to measure the remaining basal levels of DA and metabolites. After obtaining a stable output the striatum was perfused with 10 mM MPP^+ for 15 min. If the perfusion with a test compound was found to have irreversible effects on the release of DA, as shown by a small or nondetectable MPP^+-induced DA increase, the test compound was perfused in the next experiment for a shorter time and this was repeated until a perfusion duration was found which was nontoxic to the dopaminergic nerve terminals. Each experiment was at least performed in duplicate. Effects of drug perfusions on the output of DA were calculated as percentages of the basal DA release which was 4.58 ± 0.27 fmol/min (n = 123).

Intracerebral Microdialysis of Lactate. To allow the measurement of the effects of fluorescent test compounds on the in vivo formation of lactate, the recently published fluorometric method[22] was modified, with the equipment as described above, as follows: The brain dialysate effluent was mixed on-line with a reagent solution containing 5 mg/mL lactate dehydrogenase and 0.5 mM NAD^+ (Boehringer, Mannheim, GFR) in carbonate buffer (pH 9.5) at a flow rate of 15 mL/min. A polyethylene tube of about 50 cm served as the reaction coil and was directly connected to the injection valve. Every 5 min a 10-mL sample was automatically injected onto the C18 column using a 0.05 M phosphate buffer (pH 6.8) as the mobile phase. The NADH generated in the reaction coil was measured electrochemically at 650 mV. As soon as the efflux of lactate had stabilized, a test compound was perfused as a 10 mM solution in Ringer Solution for 15–150 min, and the effects on striatal lactate production were determined.

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Registry No. 1·I, 36913-39-0; 2·I, 118931-22-9; 3·I, 118931-23-0; 4·I, 39795-01-2; 5·I, 27132-46-3; 6·Br, 127382-73-4; 7·ClO_4, 127382-74-5; 7·I, 127382-75-6; 8·I, 26863-17-2; 9·I, 127382-76-7; 10·I, 127382-77-8; 11·I, 78815-42-6; 12·I, 7538-79-6; 13·I, 60684-91-5; 14·Br, 26863-23-0; 15·Cl, 26942-26-7; 16·I, 64326-91-6; 17·I, 52806-02-7; 18·I, 3947-77-1; 19·I, 3947-76-0; 20·Cl, 117897-13-9; 21·I, 70664-19-6; 22, 1131-64-2; 23·Br, 26863-24-1; 24·I, 5667-11-8; 25·I, 17350-56-0; 26·I, 10125-85-6; 27, 55-65-2; 28, 1463-28-1; 29·I, 930-73-4; 31·I, 5613-08-1; 32·I, 7630-02-6; 33·I, 6457-57-4; 34·I, 2349-55-5; 35·I, 6456-44-6; 36, 13076-43-2; 37, 56162-60-8; 38, 4685-14-7; 39, 119-65-3; 40, 91-22-5; 41, 635-46-1; 42, 491-34-9; 43, 91-21-4; 44, 1612-65-3; 45, 28289-54-5; 45·HCl, 23007-85-4; 46, 110605-92-0; 46·HCl, 71158-31-1; 47, 13100-00-0; 47·HCl, 117897-14-0; 48, 51-61-6; 48·HCl, 62-31-7; *p*-MeC_6H_4Br, 106-38-7; 2-bromotoluene, 95-46-5; 1-methyl-4-piperidone, 1445-73-4; 4-hydroxy-1-methyl-4-(2-methylphenyl)piperidine, 127382-78-9; 1-methyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine hydrochloride, 127382-79-0; 4-(2-methylphenyl)pyridine, 30456-66-7; cyclopropylmethyl bromide, 7051-34-5; 4-phenylpyridine, 939-23-1; 2-methyl-1,2,3,6-tetrahydropyridine, 14965-45-8; 6-methyl-1,2,3,6-tetrahydropyridine, 127382-80-3; 1-methyl-4-(4-methylphenyl)-1,2,3,6-tetrahydropyridine hydrochloride, 127382-81-4; 1-methyl-4-(4-methylphenyl)-1,2,3,6-tetrahydropyridine, 69675-08-7; 4-(3-cyclohexenyl)pyridine, 70644-46-1; 4-(dimethylamino)pyridine, 1122-58-3; 3-phenylpyridine, 1008-88-4; 1-bromo-2-propyne, 106-96-7; 2-phenylpyridine, 1008-89-5; 4-(hydroxymethyl)pyridine, 586-95-8.