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DEPLETION OF CARDIAC NOREPINEPHRINE IN RATS AND MICE BY 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)

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Abstract—1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a commercially available chemical reagent. Although little has been known about its biological effects, recently MPTP has been reported to cause irreversible Parkinson's disease-like symptoms in humans and in monkeys. We describe here another pharmacologic effect of MPTP, the ability to deplete cardiac norepinephrine in rats and mice. In mice, cardiac norepinephrine concentration decreased within 1 hr, was maximally depleted at 24 hr, and recovered by 4–7 days after i.p. injection of a 32 mg/kg dose of MPTP. The depletion was antagonized by desipramine pretreatment, as was norepinephrine depletion by tyramine. In rats, cardiac norepinephrine depletion by 10–30 mg/kg, i.p., doses of MPTP was accompanied by depletion of cardiac dopamine and of norepinephrine in the mesenteric artery. In rats and in mice, norepinephrine in brain was affected to a smaller degree than was norepinephrine in heart, and dopamine in brain was depleted very little if at all. In spontaneously hypertensive rats, the depletion of cardiac norepinephrine was associated with a marked antihypertensive effect. The p-hydroxy analog of MPTP did not deplete cardiac norepinephrine in rats, indicating that its possible formation as a metabolite of MPTP was not involved in the depletion of cardiac norepinephrine. These findings extend the spectrum of known pharmacologic effects of MPTP.

1 - Methyl - 4 - phenyl - 1,2,3,6 - tetrahydropyridine (MPTP) (Fig. 1) was implicated recently in reports of severe parkinsonism developing in drug abusers [1, 2]. MPTP was identified in a substance sold as a new "synthetic heroin", which was administered by intravenous injection by six individuals for durations of 4–8 days. All six developed symptoms resembling naturally occurring Parkinson's disease. The symptoms persisted without signs of spontaneous remission for at least several months and were responsive to treatment with a combination of L-dopa and carbidopa used to treat naturally occurring Parkinson's disease. MPTP was also implicated in another case of parkinsonism in a drug abuser [3], leading to the suggestion of a potential neurotoxicity of MPTP toward neurons in the substantia nigra. Recently, a case report was described of a chemist who developed Parkinson's disease after prolonged exposure to MPTP in the laboratory [4]. MPTP has been reported to deplete striatal dopamine in monkeys and to cause symptoms like those in Parkinson's disease [5].

We are describing here another pharmacologic action of MPTP, namely the ability to deplete catecholamines in heart and other peripheral tissues of mice and rats. There is no apparent relationship between this effect and the clinical symptoms of parkinsonism that have been described.

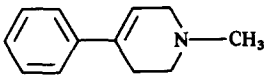

Fig. 1. Chemical structure of MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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MATERIALS AND METHODS

Male Wistar rats weighing approximately 150 g were obtained from Harlan Industries, Cumberland, IN, and male Cox standard mice weighing approximately 20 g were obtained from Laboratory Supply Co, Indianapolis, IN. In two experiments, spontaneous hypertensive rats (approximately 300 g) from Taconic Farms (Germantown, NY) were used. Arterial blood pressure was measured from a cannulated artery in pentobarbital-anesthetized rats. MPTP hydrochloride, synthesized in the Lilly Research Laboratories, was dissolved in distilled water and injected intraperitoneally. Animals were decapitated, and heart, brain, mesenteric artery and adrenal glands were removed as indicated, frozen on dry ice, and stored at −15° prior to analysis. Catecholamines were measured by high performance liquid chromatography with electrochemical detection [6]. All animals in a single experiment were killed within a 1 hr period, having been treated previously at the times indicated in each table or figure, and the frozen tissues from each experiment were analyzed in the same assay. Statistical comparisons were made by Student's *t*-test.

RESULTS

In mice, MPTP at a dose of 32 mg/kg, i.p., markedly depleted cardiac norepinephrine and had less effect on brain catecholamines (Table 1). Within 1 hr, cardiac norepinephrine was decreased significantly, and by 24 hr a maximum depletion of 84% was reached. Norepinephrine in brain was significantly but only slightly decreased at early times (28% at 1 hr), as was dopamine (14% decrease at

Table 1. Effect of MPTP on catecholamines in mouse brain and heart: time course*

Hours after MPTP injection	Catecholamines (nmoles/g) Brain norepinephrine	Brain dopamine	Heart norepinephrine
0	1.89 ± 0.06	4.64 ± 0.07	3.37 ± 0.24
1	1.36 ± 0.06†	3.99 ± 0.13†	2.01 ± 0.12†
2	1.72 ± 0.12	4.31 ± 0.13	1.96 ± 0.18†
4	1.66 ± 0.06†	4.31 ± 0.07†	0.77 ± 0.18†
24	2.13 ± 0.12	5.29 ± 0.13	0.53 ± 0.06†

* MPTP was injected i.p. at 32 mg/kg. Mean values ± standard errors for five mice per group are shown.
† Significant decrease ($P < 0.01$).

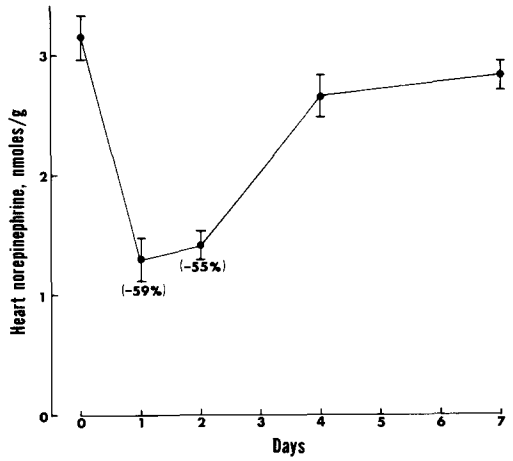

Fig. 2. Duration of heart norepinephrine depletion in mice by MPTP (32 mg/kg, i.p.). Percent changes are shown for values that differed significantly from the zero time value ($P < 0.05$). Mean values ± standard errors for five mice per group are shown.

1 hr). In these mice, hyperactivity, tremors and salivation were observed at the early times. The total duration of depletion of cardiac norepinephrine is shown in Fig. 2. Essentially maximum depletion of norepinephrine persisted at 2 days, but by 4 days no significant effect remained and by 7 days the return to normal levels of norepinephrine was almost complete.

Table 2 shows that the depletion of cardiac norepinephrine by MPTP was antagonized by pretreatment with desipramine, an inhibitor of uptake into norepinephrine neurons. Such antagonism has also been reported for the depleting action of tyramine [7], which was included for comparison in this experiment. MPTP caused 66% depletion of norepinephrine in control mice. Desipramine antagonized but did not completely prevent the effect of MPTP; norepinephrine was depleted by 36% in mice pretreated with desipramine. Tyramine decreased norepinephrine by 42% in control mice; in desipramine-pretreated mice the slight depletion by tyramine was not statistically significant from untreated controls.

Table 2, Antagonism by desipramine of the depletion of norepinephrine in mouse heart by MPTP and by tyramine*

Treatment group	Norepinephrine (nmoles/g)
None	3.14 ± 0.30
MPTP	1.07 ± 0.24†
MPTP + desipramine	2.01 ± 0.07†‡
Tyramine	1.30 ± 0.12†
Tyramine + desipramine	2.43 ± 0.24‡

* MPTP or tyramine hydrochloride (Sigma) was injected i.p. at 32 and 100 mg/kg, respectively, 2 hr before mice were killed and 30 min after desipramine hydrochloride (USV) at 20 mg/kg, i.p. Mean values ± standard errors for five mice per group are shown.
† Significant decrease ($P < 0.05$).
‡ Significant difference from corresponding group without desipramine ($P < 0.05$).

Similar depletion of cardiac norepinephrine occurred in rats (Table 3). At both doses studied (10 and 30 mg/kg), norepinephrine depletion in heart occurred within 4 hr but was greater at 24 hr. Norepinephrine was also depleted in mesenteric artery. Maximum depletion at 24 hr after the 30 mg/kg dose was 81% in heart and 82% in mesenteric

Table 3. Depletion of catecholamines in rat tissues by MPTP*

Dose (mg/kg, i.p.)	Time after injection (hr)	Norepinephrine concentration (nmoles/g tissue) Heart	Mesenteric artery	Brain	Dopamine concentration (nmoles/g tissue) Heart	Brain
0		7.40 ± 0.30	15.6 ± 1.3	2.64 ± 0.06	0.21 ± 0.02	6.61 ± 0.20
10	4	5.11 ± 0.33†	9.4 ± 0.7†	2.22 ± 0.06†	0.04 ± 0.01†	6.15 ± 0.06
	24	2.94 ± 0.19†	5.2 ± 0.5†	2.32 ± 0.05†	0.05 ± 0.02†	6.37 ± 0.11
30	4	3.32 ± 0.33†	6.3 ± 1.1†	2.09 ± 0.08†	0.06 ± 0.01†	6.46 ± 0.05
	24	1.43 ± 0.18†	2.8 ± 0.2†	2.30 ± 0.03†	0.04 ± 0.01†	6.62 ± 0.12

* Mean values ± standard errors for five rats per group are shown.
† Significant decrease ($P < 0.05$).

Table 4. Effects of MPTP in spontaneously hypertensive rats*

Dose of MPTP (mg/kg, i.p.)	Blood pressure (mm Hg)	Heart norepinephrine (nmoles/g)
0	176 ± 5	5.12 ± 0.24
1	169 ± 5	4.38 ± 0.18
3	184 ± 5	3.27 ± 0.22†
5	136 ± 10†	1.99 ± 0.99†
10	91 ± 19†	1.24 ± 0.07†

* Measurements were at 24 hr after drug administration and represent mean values ± standard errors for four rats per group.
† Significant decrease ($P < 0.05$).

artery. The depletion in brain was less pronounced and shorter in duration. There was less depletion in brain at 24 hr than at 4 hr after both doses, and the maximum depletion after the 30 mg/kg dose was only 21%. Dopamine was decreased in heart, where it presumably is present as a precursor in norepinephrine neurons, but not in brain, where it occurs mainly as a neurotransmitter in dopamine neurons.

To investigate the possible hypotensive effects of norepinephrine depletion by MPTP, we gave it to spontaneously hypertensive rats, which typically show a greater fall in blood pressure in response to hypotensive drugs than do normotensive rats (Table 4). At doses of 1, 3, 5 and 10 mg/kg, there was a dose-related depletion of cardiac norepinephrine. Associated with this depletion was a dose-related decrease in mean arterial blood pressure at the two higher doses. Table 5 shows the depletion of norepinephrine in different heart regions of spontaneously hypertensive rats. The percentage depletion ranged from 67% in the right ventricle to 90% in the left atrium.

Table 5. Depletion of norepinephrine in heart regions of spontaneously hypertensive rats*

Heart region	Norepinephrine (nmoles/g) Control	Treated
Right atrium	10.5 ± 0.9	3.1 ± 0.3†
Left atrium	11.4 ± 4.1	1.1 ± 0.6†
Right ventricle	6.1 ± 0.4	2.0 ± 0.2†
Left ventricle	5.0 ± 0.2	1.4 ± 0.1†

* Measurements were at 24 hr after administration of MPTP at 10 mg/kg, i.p., and represent mean value ± standard errors for five rats per group.
† Significant decrease ($P < 0.05$).

Because norepinephrine depletion reached maximum at 24 hr and persisted still at 2 days, despite the fact that overt effects of MPTP (CNS stimulation) lasted only an hour or so in rats, we considered the possibility that MPTP might be converted to an active metabolite that was responsible for the depletion of norepinephrine. MPTP has some structural resemblance to amphetamine, and amphetamine in rats is metabolized by para-hydroxylation. The para-hydroxy analog of MPTP might be expected not to cross the blood–brain barrier readily, and involvement of this compound in the depletion of heart norepinephrine could explain why brain catecholamines were only marginally affected. To test that idea, we injected the para-hydroxy analog of MPTP and also pretreated some rats before MPTP administration with iprindole, an inhibitor of the para-hydroxylation of amphetamine [8]. The depletion of cardiac norepinephrine by MPTP was not antagonized by iprindole but was, perhaps, slightly enhanced (Table 6). The para-hydroxy analog of MPTP was devoid of norepinephrine-depleting activity. In this experiment, adrenal epinephrine was measured as well and found to be slighly depleted by MPTP, the degree of catecholamine depletion being intermediate between that in heart and in brain.

DISCUSSION

The recent reports [1–5] of central neurotoxic actions of MPTP prompted us to report the ability of MPTP to deplete cardiac norepinephrine, although there may not be a direct relationship between these two effects. The appearance of a Parkinson's disease-like syndrome that is treatable with L-dopa in humans and monkeys apparently is due to destruction of nigrostriatal dopamine neurons in these species [3, 5], since degeneration of these neurons is believed to be the cause of spontaneously occurring parkinson's disease. Our studies with single doses of MPTP revealed little or no depletion of dopamine in brain of rats and mice, however.

MPTP did cause pronounced depletion of norepinephrine in heart and in another peripheral

Table 6. Effect of MPTP on norepinephrine in heart and brain and on epinephrine in adrenal glands in rats; influence of iprindole pretreatment and comparison of *p*-hydroxy-MPTP*

Treatment group	Heart norepinephrine (nmoles/g)	Brain norepinephrine (nmoles/g)	Adrenal epinephrine (μmoles/g)
Control	8.27 ± 0.57	2.85 ± 0.08	2.27 ± 0.20
MPTP	1.45 ± 0.08†	2.65 ± 0.05	1.62 ± 0.15†
MPTP + iprindole	0.92 ± 0.07†	2.26 ± 0.06†	1.19 ± 0.08†
p-Hydroxy-MPTP	8.11 ± 0.36	2.74 ± 0.05	2.17 ± 0.21

* MPTP and *p*-hydroxy-MPTP were injected at 32 mg/kg, i.p., 24 hr before rats were killed and 10 min after iprindole hydrochloride (10 mg/kg, i.p.). Mean values ± standard errors for five rats per group are shown.
† Significant decrease ($P < 0.05$).

sympathetically innervated tissue, the mesenteric artery. Depletion of cardiac norepinephrine was found both in rats and in mice and was associated with a marked reduction in blood pressure in spontaneously hypertensive rats. Norepinephrine was depleted to a much greater extent in heart than in brain of both species and to a greater extent than was adrenal epinephrine in rats. The depletion of cardiac norepinephrine after a single dose of MPTP was long-lasting but was reversible and, like the depletion of tyramine, was apparently dependent upon the membrane uptake pump in that it was antagonized by desipramine, an inhibitor of that uptake pump.

The lack of marked depletion of catecholamines in brain is probably not due to poor penetration of MPTP into brain, since visible signs of central excitation (tremors, head-searching movements, exophthalmos, piloerection and tail erection) were present within 5 min after injection of the 32 mg/kg, i.p., dose into rats. Within a couple of hours, the animals returned to normal appearance. A higher dose (100 mg/kg, i.p.) was lethal to rats, five out of five rats treated with this dose dying with convulsions within 15 min. We have not developed an assay for MPTP to permit measurement of its concentration in brain or its rate of disappearance from tissues. The enhancement of the effects of MPTP by iprindole would be consistent with ring hydroxylation being a pathway of MPTP metabolism in rats, as is the case for amphetamine. The significance of the depletion of dopamine in heart by MPTP is not known. The presence of low concentrations of dopamine in heart and other peripheral tissues has led some workers to postulate the existence of peripheral dopaminergic nerves [9], in which case our findings would suggest that these nerves are affected the same as noradrenergic nerves by MPTP. On the other hand, the low concentrations of dopamine may be present in norepinephrine-forming nerves as a precursor, and the depletion by MPTP may simply be another manifestation of the effects of that drug on these neurons.

Our findings, together with the recent publications discussed above, reveal that MPTP has potent pharmacologic and toxic effects, some differences in the effects possibly occurring among species.

REFERENCES

1. J. W. Langston, P. Ballard, J. W. Tetrud and I. Irwin, *Science* **219**, 979 (1983).
2. P. Ballard, J. W. Langston, J. Tetrud and R. S. Burns, *Neurology* **33** (Suppl. 2), 90 (1983).
3. G. C. Davis, A. C. Williams, S. P. Markey, M. H. Ebert, E. D. Caine, C. M. Reichert and I. J. Kopin, *Psychiat. Res.* **1**, 249 (1979).
4. J. W. Langston and P. A. Ballard, Jr., *New Engl. J. Med.* **309**, 310 (1983).
5. R. S. Burns, C. C. Chiueh, S. P. Markey, M. H. Ebert, D. M. Jacobowitz and I. J. Kopin, *Proc. natn. Acad. Sci. U.S.A.* **80**, 4546 (1983).
6. R. W. Fuller and K. W. Perry, *Biochem. Pharmac.* **26**, 2087 (1977).
7. B. B. Brodie, E. Costa, A. Groppetti and C. Matsumoto, *Br. J. Pharmac.* **34**, 648 (1968).
8. J. J. Freeman and F. Sulser, *J. Pharmac. exp. Ther.* **307**, (1972).
9. Z. Lackovic and N. H. Neff, *Life Sci.* **32**, 1665 (1983).