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COMPARISON OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) AND 1-METHYL-4-PHENYLPYRIDINIUM (MPP^+) EFFECTS ON MOUSE HEART NOREPINEPHRINE

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Abstract—MPP^+ (1-methyl-4-phenylpyridinium) mimicked MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in producing marked, dose-related depletion of cardiac norepinephrine after a single oral or subcutaneous dose in mice. MPP^+ was approximately 4-fold more potent than MPTP in depleting norepinephrine, but the onset of depletion was not faster for MPP^+ than for MPTP. The time courses of the effects of both compounds were similar to that for 6-hydroxydopamine, with maximum depletion occurring at 1 day, partial recovery at 2 and 4 days, and full recovery of norepinephrine concentrations at 1 week. Desipramine, over a dose range that completely prevented the depletion of cardiac norepinephrine by 6-hydroxydopamine at 24 hr, did not prevent cardiac norepinephrine depletion by either MPP^+ or MPTP. In a short duration experiment, one or two doses of desipramine also failed to prevent heart norepinephrine depletion by MPP^+ or by MPTP, although a slight antagonism was found. EXP 561 (4-phenylbicyclo[2,2,2]octan-1-amine hydrochloride monohydrate), another uptake inhibitor with possibly longer duration of action, also did not protect against norepinephrine depletion by a single dose of MPP^+ or MPTP at a dose that prevented norepinephrine depletion by 6-hydroxydopamine. In mice given four daily doses of MPTP, EXP 561 prevented the depletion of norepinephrine in the frontal cortex and of dopamine in the striatum but not the depletion of norepinephrine in heart or spleen. Thus, both MPTP and MPP^+ deplete norepinephrine in mouse heart, and this effect of the two compounds is resistant to antagonism by uptake inhibitors that antagonize the effects of MPTP on brain catecholamines.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has become a topic of intense neurochemical research since it was discovered to cause Parkinsonian symptoms in human drug abusers who inadvertently self-administered it [1]. In various animal species, MPTP causes marked and persistent depletion of striatal dopamine [2–4]. The depletion of striatal dopamine is prevented by inhibition of monoamine oxidase type B (MAO-B), the enzyme that oxidizes MPTP to MPP^+ (1-methyl-4-phenylpyridinium), or by inhibition of MPP^+ uptake by the membrane uptake carrier on dopamine neurons [5–7]. MPP^+ does not cross the blood–brain barrier (R. J. D'Amato, personal communication, cited with permission) and does not deplete striatal dopamine when given systemically [8], but it does deplete striatal dopamine when applied directly into the striatum, the substantia nigra or the median forebrain bundle [9, 10], although it also produces nonspecific neural damage near the site of injection [11].

We have reported previously that MPTP depletes heart norepinephrine in mice and in rats [12] and that this depletion, unlike the depletion of dopamine in the striatum or of norepinephrine in the frontal cortex, is not prevented by MAO-B inhibitors [13]. The present experiments were done to characterize more fully the effects of MPTP and of MPP^+ on cardiac norepinephrine in mice. Some characteristics of the effects on heart norepinephrine contrasted with the effects of MPTP on brain catecholamines, e.g. pronounced depletion of heart norepinephrine occurred after a single dose of MPTP or MPP^+ and was not prevented by uptake inhibitors.

MATERIALS AND METHODS

Male CRL/CFW mice weighing 20–30 g (Charles River Breeding Laboratories, Wilmington, MA) were given drugs by s.c. injection or by oral gavage. Tissues were removed from decapitated mice, frozen on dry ice, and stored at −15° prior to catecholamine analysis by liquid chromatography with electrochemical detection [14]. 6-Hydroxydopamine hydrobromide was purchased from the Regis Chemical Co., Morton Grove, IL. Desipramine hydrochloride was a gift from USV Laboratories, Tarrytown, NY, and EXP 561, 4-phenylbicyclo[2,2,2]octan-1-amine hydrochloride monohydrate, was a gift from E. I. du Pont de Nemours & Co., Wilmington, DE. MPTP hydrochloride and MPP^+ iodide were synthesized in the Lilly Research Laboratories. Statistical analyses were done by analysis of variance using Tukey's test for significant differences.

RESULTS

Figure 1 compares the potencies of MPTP and MPP^+ in depleting heart norepinephrine after their

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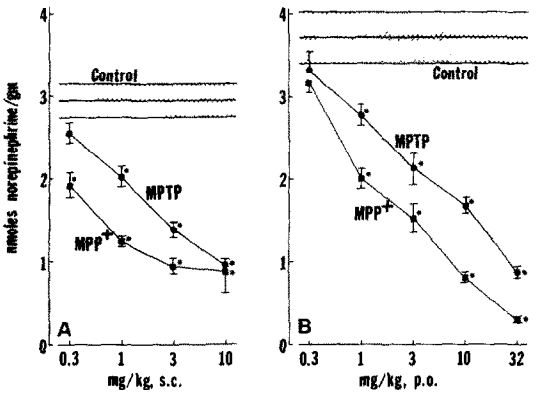

Fig. 1. Comparative potencies of MPTP and MPP+ in depleting heart norepinephrine in mice: Dose-dependent depletion of heart norepinephrine 24 hr after administration of (A) subcutaneous or (B) oral doses of MPTP or MPP+. Mean values ± SE for five mice per group are shown. Asterisks indicate significant differences from the control group ($P < 0.05$).

administration by subcutaneous injection (Fig. 1A) or by oral gavage (Fig. 1B). Both MPTP and MPP+ caused dose-dependent decreases in heart norepinephrine concentration when given subcutaneously or orally. MPP+ was slightly more potent than MPTP by both routes of administration. MPTP was slightly more potent when given subcutaneously than when given orally; the same was true with MPP+ at low doses, although the 10 mg/kg dose did not produce a greater effect subcutaneously than orally. ED_{50} Values were estimated to be 2.6 and 0.7 mg/kg, respectively, for MPTP and MPP+ given subcutaneously, and 6.2 and 1.5 mg/kg, respectively, for MPTP and MPP+ given orally.

Figure 2 compares the rates of decline in norepinephrine concentration during the first 4 hr after s.c. injection of MPTP (20 mg/kg) or MPP+ (10 mg/kg), doses chosen to produce similar degrees of norepinephrine depletion. The effect of MPP+ was statistically significant even at 30 min, whereas the effect of MPTP was not significant until 1 hr post-administration. However, there was overall no clearly faster onset of effect with MPP+ than with MPTP, as might have been expected if MPTP had to be converted to MPP+ in order to deplete norepinephrine.

Figure 3 shows the total duration of heart norepinephrine depletion by MPTP (20 mg/kg, s.c.) and MPP+ (10 mg/kg, s.c.) in mice. The maximum depletion occurred on day 1, but depletion persisted on days 2 and 4 after a single subcutaneous injection of either agent. By day 7, heart norepinephrine concentration had returned to control values. 6-Hydroxydopamine HBr (7 mg/kg, i.p.) was included in this experiment; its depletion of heart norepinephrine followed a time course remarkably similar to that for MPTP and MPP+.

The effect of pretreatment with an uptake inhibitor, desipramine, on norepinephrine depletion by 6-hydroxydopamine, MPTP and MPP+ is shown in Fig. 4. Desipramine caused a dose-related antagonism of norepinephrine depletion by 6-hydroxydopamine but had no significant effect on norepinephrine depletion by MPTP or by MPP+. The possibility was considered that a short duration of action of desipramine might permit it to antagonize the effects of 6-hydroxydopamine, which persists for a very short time in tissues [15], but not allow it to antagonize the effects of MPTP or of MPP+ if they persist for several hours. In a second experiment, norepinephrine was measured at a shorter time after MPTP or MPP+ administration (4 hr), and a second injection of desipramine was given 2 hr after MPTP or MPP+ administration. Table 1 shows that under these conditions desipramine still failed to prevent heart norepinephrine depletion by either MPTP or MPP+. A slight but statistically significant antagonism of MPTP was produced by desipramine after either one or two doses, and a slight but statistically significant antagonism of MPP+ was produced by two doses but not by a single dose of desipramine.

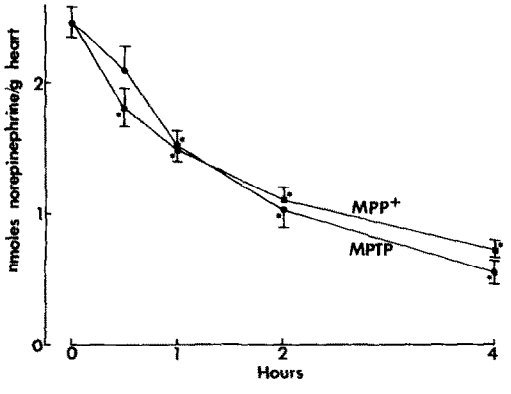

Fig. 2. Rate of decline in heart norepinephrine concentration after equieffective s.c. doses of MPTP (20 mg/kg) or MPP+ (10 mg/kg) in mice. Mean values ± SE for five to six mice per group are shown. Asterisks indicate significant differences from the control group ($P < 0.05$).

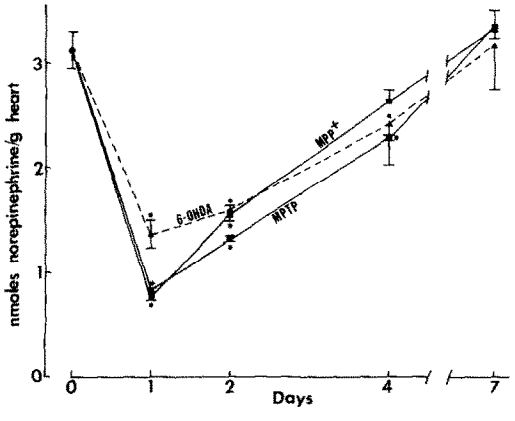

Fig. 3. Time courses of the decreases in heart norepinephrine produced by MPTP, MPP+ or 6-hydroxydopamine in mice. MPTP HCl (20 mg/kg, s.c.), MPP+ iodide (10 mg/kg, s.c.) or 6-hydroxydopamine HBr (7 mg/kg, i.p.) was injected at zero time. Mean values ± SE for five mice per group are shown. Asterisks indicate significant differences from the control group ($P < 0.05$).

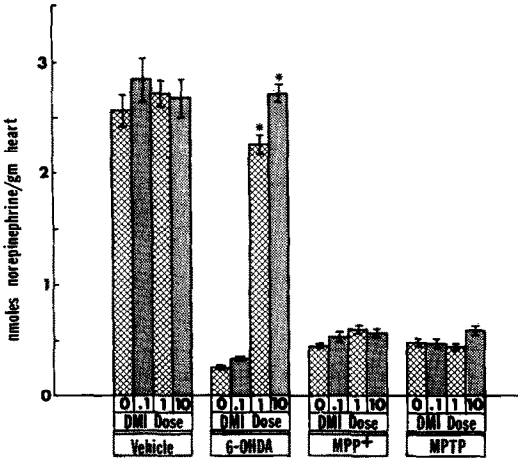

Fig. 4. Effect of an uptake inhibitor, desipramine, on the depletion of heart norepinephrine in mice by MPTP, MPP+ or 6-hydroxydopamine. Desipramine was injected at 0.1, 1 or 10 mg/kg, i.p., 15 min before MPTP (20 mg/kg, s.c.), MPP+ (10 mg/kg, s.c.) or 6-hydroxydopamine (10 mg/kg, s.c.). Mean values ± SE for five to seven mice per group are shown. Asterisks indicate significant antagonism of norepinephrine depletion ($P < 0.05$).

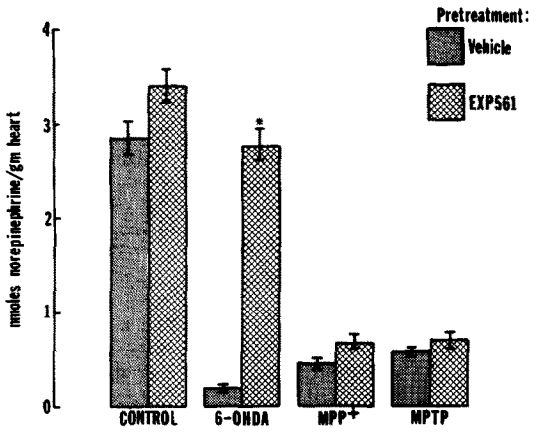

Fig. 5. Effect of an uptake inhibitor, EXP 561, on the depletion of heart norepinephrine in mice by MPTP, MPP+ or 6-hydroxydopamine. EXP 561 was injected at 5 mg/kg, i.p., 15 min before MPTP (20 mg/kg, s.c.), MPP+ (10 mg/kg, s.c.) or 6-hydroxydopamine (10 mg/kg, s.c.), and mice were killed 24 hr later. Mean values ± SE for five mice per group are shown. The asterisk indicates significant antagonism of norepinephrine depletion ($P < 0.05$).

Another uptake inhibitor thought to have a longer duration of action [16] also was examined. Figure 5 shows that EXP 561 completely prevented heart norepinephrine depletion by 6-hydroxydopamine but did not alter heart norepinephrine depletion by either MPTP or MPP+. In another experiment, four daily doses of MPTP were given, and catecholamines were measured in brain as well as in heart and spleen 24 hr after the last dose (Table 2). Norepinephrine was depleted in frontal cortex, heart and spleen, and dopamine was depleted in the striatum. Pretreatment with EXP 561 prior to each dose of MPTP prevented depletion of brain catecholamines but did not alter the depletion of norepinephrine in heart or spleen.

DISCUSSION

The present experiments extend our earlier observation [13] that MPP+ shares with MPTP the ability to deplete heart norepinephrine in mice. MPP+ was slightly more potent than MPTP in producing this effect when the compounds were injected orally or subcutaneously. In contrast, MPP+ injected systemically does not deplete brain catecholamines [8], because it does not cross the blood–brain barrier (R. J. D'Amato, personal communication of results with tritiated MPP+, cited with permission). The efficacy of MPP+ given orally indicates that this quaternary compound can be absorbed orally.

The depletion of brain catecholamines after MPTP injection depends on conversion to MPP+. The depletion of heart norepinephrine does not appear to depend upon conversion to MPP+, since MAO inhibitors do not prevent the depletion of heart norepinephrine by MPTP [13]. Although MPP+ was slightly more potent than MPTP in depleting heart norepinephrine, it did not cause any distinctly faster depletion as might be expected if MPTP had to be metabolized to MPP+ in order to produce this effect.

Table 1. Inability of desipramine (DMI) to prevent the depletion of norepinephrine in mouse heart after a single dose of MPTP or MPP+

	Heart norepinephrine (nmol/g)		
Treatment	No pretreatment	DMI one dose	DMI two doses
Vehicle	3.04 ± 0.17	2.88 ± 0.08	2.76 ± 0.08
MPTP	1.13 ± 0.09* (−63%)	1.57 ± 0.15*† (−45%)	1.88 ± 0.10*† (−32%)
MPP+	1.24 ± 0.06* (−59%)	1.14 ± 0.06* (−60%)	1.49 ± 0.05*† (−46%)

MPTP HCl (20 mg/kg, s.c.) or MPP+ iodide (10 mg/kg, s.c.) was injected 4 hr before rats were killed. Desipramine (10 mg/kg, i.p.) was injected once (15 min before MPTP or MPP+) or twice (15 min before and 2 hr after MPTP or MPP+). Mean values ± SE for five mice per group are shown. Values in parentheses represent per cent decrease relative to corresponding vehicle control.

* Significant difference from control ($P < 0.05$).

† Significant difference from same treatment group with no DMI ($P < 0.05$).

Table 2. Effect of EXP 561 on peripheral and central catecholamine depletion by repeated doses of MPTP in mice

Treatment group	Catecholamines (nmol/g)			
	Striatal DA	Cortical NE	Heart NE	Spleen NE
Control	68.2 ± 2.9	1.03 ± 0.05	3.00 ± 0.12	2.13 ± 0.25
MPTP alone	24.0 ± 1.8* (−65%)	0.79 ± 0.05* (−24%)	0.41 ± 0.05* (−86%)	0.50 ± 0.12* (−76%)
MPTP + EXP 561	62.8 ± 3.8†	1.20 ± 0.06†	0.50 ± 0.02* (−83%)	0.63 ± 0.06* (−70%)
EXP 561 alone	70.6 ± 3.0	1.14 ± 0.05	2.60 ± 0.07*	1.84 ± 0.16

MPTP HCl (20 mg/kg, s.c.) was injected once daily for 4 days, and mice were killed 24 hr after the last dose. EXP 561 (5 mg/kg, i.p.) was injected 15 min before each dose of MPTP. Mean values ± SE for five mice per group are shown. Values in parentheses represent per cent decrease relative to control group.

* Significant difference from control group ($P < 0.05$).

† Significant difference from MPTP alone ($P < 0.05$).

The time course of heart norepinephrine depletion by either MPP^+ or MPTP resembled closely the time course of norepinephrine depletion by 6-hydroxydopamine, a known neurotoxin for noradrenergic neurons [17]. With all three agents, norepinephrine concentration in mouse heart had returned to control values within 7 days after a single injection. The recovery of heart norepinephrine levels was unanticipated since 6-hydroxydopamine-induced depletion of norepinephrine in brain persists for much longer times [18, 19].

Both MPP^+ and MPTP differed from 6-hydroxydopamine in that their depletion of heart norepinephrine was resistant to antagonism by inhibitors of the membrane uptake carrier on norepinephrine neurons. Desipramine at doses that completely prevented 6-hydroxydopamine action did not alter the depletion of heart norepinephrine by MPP^+ or by MPTP. The inability of desipramine to prevent heart norepinephrine depletion at 24 hr after MPP^+ or MPTP injection theoretically could result from a short duration of action of desipramine. 6-Hydroxydopamine has a short half-life in biological tissue [15], so uptake inhibition for a short period of time would prevent its effects. If MPTP and MPP^+ persisted for a longer time in heart, then uptake inhibition for a longer time might be necessary to prevent their norepinephrine-depleting effects. However, when heart norepinephrine was measured only 4 hr after MPP^+ or MPTP injection and two injections of desipramine were given, desipramine still failed to prevent norepinephrine depletion. A slight antagonism of the depletion was found in this experiment, consistent with a partial antagonism of heart norepinephrine depletion by MPTP seen in an earlier study involving a higher dose of desipramine [12].

EXP 561, a potent inhibitor of norepinephrine uptake (also serotonin and dopamine uptake) with a relatively long duration of action [16], also failed to prevent heart norepinephrine depletion by MPP^+ or MPTP. In contrast, the central effects of MPTP on catecholamines in the striatum and frontal cortex were fully antagonized by EXP 561. Thus, these data suggest that the depletion of cardiac norepinephrine by MPTP or by MPP^+ may not require active accumulation by the uptake carrier on norepinephrine neurons.

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