J. Pharm. Pharmacol. 1987, 39: 667–669
Communicated February 17, 1987

Persistent depletion of striatal dopamine and its metabolites in mice by TMMP, an analogue of MPTP

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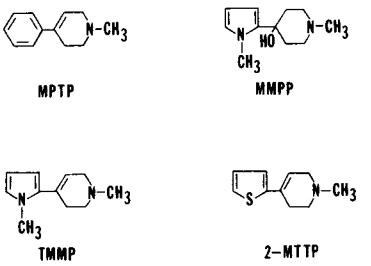
TMMP (1-methyl-4-[methylpyrrol-2-yl]-1,2,3,6-tetrahydropyridine) mimicked MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in causing persistent depletion of striatal dopamine and its metabolites one week after the last of four daily subcutaneous injections in mice. MMPP (1-methyl-4-[1-methylpyrrol-2-yl]-4-piperidinol) produced no depletion of dopamine or its metabolites under these conditions. None of the three compounds affected dopamine or its metabolites when administered orally. TMMP was even more rapidly oxidized by type B monoamine oxidase in-vitro than was MPTP, but MMPP was a very poor substrate for the enzyme. The lack of neurotoxicity of MMPP toward nigrostriatal dopamine neurons when it was given orally or subcutaneously to mice contrasts with previously reported results in monkeys, in which case MMPP was reported to be neurotoxic.

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, has been reported to cause parkinsonian-like symptoms accompanied by destruction of dopaminergic neurons in the substantia nigra in humans (Langston et al 1983) and in non-human primates (Burns et al 1983). In mice, MPTP causes persistent loss of striatal dopamine and its metabolites, but no parkinsonian-like symptoms (Heikkila et al 1984a). Several structural analogues of MPTP have been examined, and some have been found to mimic MPTP in causing persistent depletion of striatal dopamine in mice or other species. Wilkening et al (1986) have recently reported that MMPP (1-methyl-4-[1-methylpyrrol-2-yl]-4-piperidinol) caused parkinsonian symptoms and lesions of neurons in the substantia nigra in monkeys, although another analogue (TMMP, 1-methyl-4-[methylpyrrol-2-yl]-1,2,3,6-tetrahydropyridine) was inactive. TMMP has some structural resemblance to 2-MTTP, another heterocyclic analogue of MPTP that we recently reported to cause persistent depletion of striatal dopamine in mice (Fuller et al 1986), and to 2′-methyl-MPTP, which is more potent than MPTP in producing persistent depletion of striatal dopamine in mice (Youngster et al 1986). We, therefore, compared TMMP and MMPP with MPTP in mice and report here the observation that TMMP but not MMPP caused persistent depletion of striatal dopamine.

Methods

Male CRL/CFW mice weighing 20–30 g (Charles River Breeding Laboratories, Portage, MI) were given MPTP HCl (synthesized by Dr David W. Robertson in the Lilly Research Laboratories), MMPP or TMMP (both provided by E. I. duPont de Nemours, Wilmington, DE) either by subcutaneous injection or by oral gavage. All compounds were dissolved in distilled water (4 mg mL^{-1}) and were given at a dose of 20 mg kg^{-1}. No death from the drugs occurred. Four daily doses of each drug were given, and mice were killed one week after the last dose; this treatment has been found to be suitable for demonstrating neurotoxicity of MPTP analogues (Fuller et al 1987). Brains were quickly removed and dissected, then striata were frozen on dry ice and stored at −15 °C before analysis. Dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by liquid chromatography with electrochemical detection (Fuller & Perry 1977; Perry & Fuller 1979). Hydrogen peroxide formation associated with the oxidation of MPTP by a mouse brain mitochondrial preparation in-vitro was measured by a modification of the method of Szutowicz et al (1984). Hydrogen peroxide formation was measured colorimetrically after addition of the substrates at concentrations of 1 to 5 mM. Mitochondria containing 1·65 mg protein were added to each reaction vessel, and incubation was at 37 °C for 60 min.



Results

Table 1 shows that MPTP and TMMP markedly depleted dopamine and its metabolites in mouse striatum one week after the last of four daily subcutaneous injections. The percentage depletion by TMMP of dopamine and each of its two metabolites was slightly greater than the percentage depletion by MPTP. No significant depletion of dopamine, DOPAC or HVA was produced by MMPP. Since these findings differed from those of Wilkening et al (1986), who had given the

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Table 1. Striatal concentrations of dopamine and its metabolites one week after the last of four daily subcutaneous injections of MPTP, MMPP or TMMP in mice.

Treatment group	Striatal dopamine and metabolites, nmol g^{-1}		
	Dopamine	DOPAC	HVA
Control	64·4 ± 1·3	5·70 ± 0·05	6·03 ± 0·10
MPTP	32·4 ± 1·6* (−50%)	3·18 ± 0·15* (−44%)	4·45 ± 0·21* (−26%)
MMPP	62·6 ± 0·7	5·77 ± 0·19	6 44 ± 0·10
TMMP	24·7 ± 1·2* (−62%)	2·70-p 0·15* (−53%)	3·92 ± 0·09* (−35%)

* Significant change from control ($P < 0·05$).

All compounds were injected at 20 mg kg^{-1} s.c. Mice were killed one week after four daily injections of each of the compounds. Mean values ± standard errors for 6 mice per group are shown.

Table 2. Striatal concentrations of dopamine and its metabolites one week after the last of four daily oral doses of MPTP, MMPP or TMMP in mice.

Treatment group	Striatal dopamine and metabolites, nmol g^{-1}		
	Dopamine	DOPAC	HVA
Control	66·7 ± 2·0	5·11 ± 0·29	6·37 ± 0·21
MPTP	72·1 ± 1·4	5·30 ± 0·16	6·26 ± 0·15
MMPP	69·4 ± 1·4	4·94 ± 0·15	5·91 ± 0·13
TMMP	68·9 ± 2·0	5·32 ± 0·27	7·11 ± 0·26

All compounds were given by oral gavage at 20 mg kg^{-1}. Mice were killed one week after four daily doses of each of the compounds. Mean values ± standard errors for 8 mice per group are shown.

compounds orally to monkeys, we did a second experiment in which the compounds were given orally to mice. Table 2 shows that none of the three compounds produced any significant depletion of dopamine or its metabolites when given orally. MPTP is a substrate for monoamine oxidase (MAO) type B (Chiba et al 1984). Fig. 1 compares the oxidation in-vitro of MPTP and its analogues by mouse brain mitochondrial MAO. TMMP was a better substrate than was MPTP, whereas MMPP had little or no detectable substrate activity. Selegiline (L-Deprenyl) (10^{-5} M) inhibited the oxidation of TMPP by 74% and inhibited the oxidation of MPTP by 82%. Since selegiline is a selective MAO-B inhibitor, the results indicate that the oxidation of both substances occurred through the action of MAO-B present in the mitochondrial preparation.

Discussion

Our results with TMMP, the analogue with the greatest structural resemblance to MPTP, agree with those of Wilkening et al (1986) in that no neurotoxicity was found after oral administration of the compound. Our results reveal, however, that TMMP did cause persistent depletion of striatal dopamine and its metabolite when it was injected subcutaneously. We have found no neurotoxicity to striatal dopamine terminals in CRL/

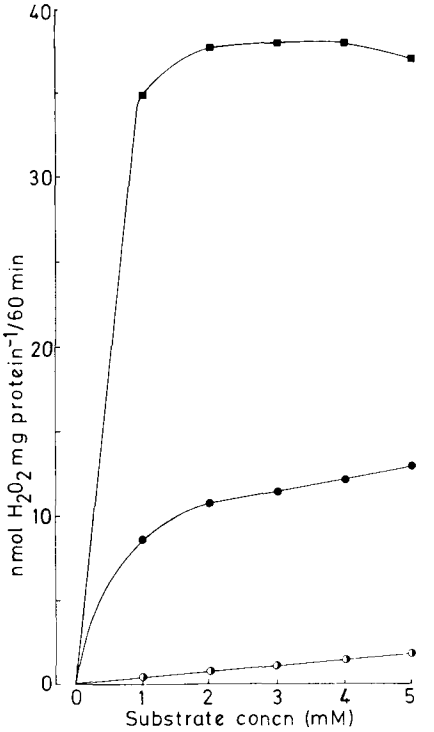

Fig. 1. Oxidation of TMMP (■), MPTP (●) and MMPP (◑) by mouse brain mitochondrial MAO in-vitro.

CFW mice given MPTP orally, even after doses of 160 mg kg^{-1}, a maximum non-lethal dose (Fuller & Hemrick-Luecke 1987). Higher doses of TMMP were not tested due to our limited supply of the compound.

The lack of oral efficacy of TMMP in monkeys and in mice probably can be explained by its ability to be oxidized so readily. Like MPTP, it may be converted so extensively to the pyridinium metabolite or to other metabolites following oral administration that circulating levels of the parent compound are too low to enter the brain and cause neurotoxicity. The pyridinium metabolite would not be expected to cross the blood-brain barrier. For instance, MPP + (1-methyl-4-phenylpyridinium), the metabolite of MPTP, does not deplete striatal dopamine in mice when it is given systemically (Leavitt et al 1986).

The discrepancy between our results in mice and those of Wilkening et al (1986) in monkeys is with MMPP. They reported neurotoxicity to striatal dopamine neurons when MMPP was administered orally or parenterally to monkeys, whereas we found no depletion of striatal dopamine in mice. Our data are consistent with the relative inability of MMPP to be oxidized by brain MAO. The very low rate of oxidation of MMPP by mouse brain MAO may account for its lack of effect on striatal dopamine neurons, since the neurotoxic effect of MPTP requires its metabolism by

MAO-B (Heikkila et al 1984b). The discrepant findings may indicate a species difference in the ability of MMPP to be converted to a neurotoxic metabolite. Perhaps in the experiments of Wilkening et al (1986), in monkeys MMPP underwent enzymatic or non-enzymatic dehydration, to form TMMP. Currently there is no evidence that mice or monkeys would more accurately predict neurotoxic potential of MPTP-like compounds in man.

REFERENCES

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M., Kopin, I. J. (1983) Proc. Nat. Acad. Sci. 80: 4546–4550

Chiba, K., Trevor, A., Castagnoli, N., Jr. (1984) Biochem. Biophys. Res. Commun. 120: 574–578

Fuller, R. W., Perry, K. W. (1977) Biochem. Pharmacol. 26: 2087–2090

Fuller, R. W., Hemrick-Luecke, S. K. (1987) Ibid. 36: 789

Fuller, R. W., Robertson, D. W., Hemrick-Luecke, S. K. (1986) Ibid. 35: 143–144

Fuller, R. W., Robertson, D. W., Hemrick-Luecke, S. K. (1987) J. Pharmacol. Exp. Ther. 240: 415–420

Heikkila, R. E., Hess, A., Duvoisin, R. C. (1984a) Science 224: 1451–1453

Heikkila, R. E., Manzino, L., Cabbat, F. S., Duvoisin, R. C. (1984b) Nature 311: 467–469

Langston, J. W., Ballard, P., Tetrud, J. W., Irwin, I. (1983) Science 219: 979–982

Leavitt, M. L., Gittings, M. L., Hemrick-Luecke, S. K., Robertson, D. W., Fuller, R. W. (1986) in: Markey, S. P., Castagnoli, N., Jr., Trevor, A. J., Kopin, I. J. (eds) MPTP: a neurotoxin producing a Parkinsonian Syndrome. Academic press, Orlando, p 581

Perry, K. W., Fuller, R. W. (1979) Soc. Neurosci. Abstr. 5: 348

Szutowicz, A., Kobes, R. D., Orsulak, P. J. (1984) Anal. Biochem. 138: 86–94

Wilkening, D., Vernier, V. G., Arthaud, L. E., Treacy, G., Kenney, J. P., Nickolson, V. J., Clark, R., Smith, D. H., Smith, C., Boswell, G. (1986) Brain Res. 368: 239–246

Youngster, S. K., Duvoisin, R. C., Hess, A., Sonsalla, P. K., Kindt, M. V., Heikkila, R. E. (1986) Eur. J. Pharmacol. 122: 283–287

J. Pharm. Pharmacol. 1987, 39: 669–672
Communicated March 31, 1987

Gastric ulcerogenicity of non-steroidal anti-inflammatory drugs in mice with mucosa sensitized by cholinomimetic treatment

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A novel technique is described for the assay of acute gastric irritancy of non-steroidal anti-inflammatory drugs (NSAIDs) in mice in which (a) the gastric mucosa is sensitized to the irritant actions of the drugs by co-administration of bethanechol chloride to increase acid and pepsin production, and (b) the area and number of haemorrhagic lesions in the glandular mucosa is measured quantitatively by visual image analysis. The technique has been used to assess the acute gastric irritancy of 20 NSAIDs in mice. In relation to published values for their acute and chronic anti-inflammatory activities, drugs with low relative gastric irritancy (e.g. carprofen, chloroquine, diclofenac, fenbufen, tenoxicam, tilomisole) were differentiated from the drugs of higher relative irritancy.

Various approaches are used in the assay of acute gastric irritancy of ulcerogenicity of non-steroidal anti-inflammatory drugs (NSAIDs) in laboratory animals, each technique having merits and disadvantages (Rainsford & Whitehouse 1977; Menassé 1979; Ghanayem & Ahmed 1982; Dearden & Nicholson 1984; Rainsford 1981, 1984, 1985a, 1987a, b; Whitehouse et al 1984; Szabo et al 1985). That most frequently used is the counting or grading of haemorrhagic lesions visible about 1–6 h after oral or parenteral administration of NSAIDs to fasted rats (Menassé 1979; Rainsford 1984). This method, while simple, yields data with high error and variability (Rainsford 1987b). To improve the sensitivity of lesion detection and reduce data error, while at the same time employing systems relevant to the therapeutic situation, some authors have subjected rats to cold, restraint, or inflammatory stress conditions to enhance the gastric irritancy of NSAI drugs (Shriver et al 1977; Rainsford 1978, 1981, 1987b; Dearden & Nicholson 1984; Whitehouse et al 1984). However, these procedures expose animals to stressful situations.

An approach to overcome these is to mimic the stressful reactions in the stomach by means of a cholinomimetic agent thereby enhancing mucosal sensitivity to the gastric irritancy of drugs such as NSAIDs. Muscarinic agents (e.g. bethanechol chloride) stimulate the secretion of acid and pepsin (Magee et al 1985) which have actions in NSAID- and stress gastric ulcerogenesis (Rainsford 1978, 1987b). Hence, in the present study the gastric irritancy of a range of NSAI drugs in mice treated with the muscarinic agent, bethanechol chloride, has been evaluated. The method used mice for improved economy (cf. rats) and their gastric lesions were quantified by magnified image analysis.

Methods

Animal procedures. The studies were in female MF1