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PERSISTENT DEPLETION OF STRIATAL DOPAMINE IN MICE BY 1-METHYL-4-(2-THIENYL)-1,2,3,6-TETRAHYDROPYRIDINE (MTTP)

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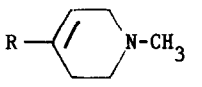
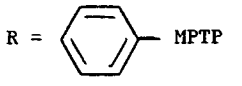
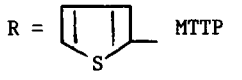
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been implicated as a cause of Parkinsonian symptoms in drug abusers who self-administered the compound when it was present as a contaminating byproduct in a sample of a narcotic drug (1). MPTP is neurotoxic to striatal dopamine neurons in primates (2,3) and in rodents (4-6). Administration of four or even fewer daily injections of MPTP to mice results in persistent depletion of striatal dopamine and its metabolites. Moreover, this regimen produces a loss of dopamine uptake capacity in striatum accompanied by histologic evidence of nerve cell loss in the substantia nigra (4-6). Not many structural analogs of MPTP have yet been examined for their ability to be neurotoxic to striatal dopamine neurons *in vivo* (7,8), but results to date indicate that small molecular changes in MPTP can obliterate neurotoxicity. Here we report that MTTP, an analog of MPTP with the phenyl group replaced by a 2-thienyl group, does produce persistent depletion of striatal dopamine and its metabolites in mice, though with slightly less potency than MPTP itself. MTTP appears to be the first heterocyclic analog of MPTP reported to have neurotoxic potential.







MATERIALS AND METHODS: Male CRL/CFW mice from Charles River Breeding Laboratories, Wilmington, MA, received daily s.c. injections of MPTP hydrochloride (20 mg/kg) or of MTTP hydrochloride (20, 40 or 80 mg/kg) for 4 days. Seven days after the last injection, mice were killed. Brains were removed rapidly; striata were dissected, frozen on dry ice, and then stored at -15° prior to analysis. Dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as serotonin and 5-hydroxyindoleacetic acid, were determined by liquid chromatography with electrochemical detection (9,10).

RESULTS: Table 1 shows striatal concentrations of dopamine, DOPAC and HVA in control mice and in mice treated with MPTP or MTTP. MPTP at a dose of 20 mg/kg produced 57% depletion of dopamine, 56% depletion of DOPAC and 39% depletion of HVA 7 days after the last of 4 daily injections. The same dose, 20 mg/kg, of MTTP did not cause any significant effects on dopamine or its metabolites, nor did a higher dose, 40 mg/kg. However, the 80 mg/kg dose of MTTP depleted dopamine, DOPAC and HVA, the percentage changes being a little less than after the 20 mg/kg dose of MPTP. Apparently MTTP can produce the same effects as MPTP on striatal dopamine neurons in mice but is slightly less than one-fourth as potent as MPTP. Serotonin and 5-hydroxyindoleacetic acid concentrations measured in striatum were not changed significantly by MPTP or by any of the doses of MTTP (data not shown).

Table 1. Persistent depletion of striatal dopamine and its metabolites in mice by MPTP and by MTTP

Treatment group	Striatal dopamine and metabolites (nmoles/g)		
	Dopamine	DOPAC	HVA
Control	74.5 ± 2.8	8.12 ± 0.14	6.35 ± 0.20
MPTP (20)	32.4 ± 2.7* (-57%)	3.61 ± 0.31* (-56%)	3.90 ± 0.21* (-39%)
MTTP (20)	72.5 ± 3.1	7.86 ± 0.25	6.25 ± 0.21
MTTP (40)	75.8 ± 2.8	8.28 ± 0.38	6.32 ± 0.25
MTTP (80)	49.6 ± 5.7* (-33%)	4.78 ± 0.38* (-41%)	5.01 ± 0.45* (-21%)

Mean values for 8 mice per group (6 in the MPTP group) are shown with standard errors. Asterisks indicate significant ($P<0.05$) differences from control group. Numbers in parentheses in the left column indicate mg/kg doses, and in the right columns indicate percentage changes for statistically significant effects.

DISCUSSION: Considerable evidence suggests that MPTP is not intrinsically toxic but functions as a protoxin: two biochemical interventions appear to be necessary for the neurotoxic effect of MPTP on striatal dopamine neurons to be expressed. First is the oxidation of MPTP by monoamine oxidase type B to form MPP+ (1-methyl-4-phenylpyridinium) (11), and the second is the accumulation of MPP+ by dopamine neurons through the action of the membrane uptake carrier (12). Heikkila and colleagues (8) reported that some analogs of MPTP are readily oxidized by MAO-B whereas others are poor substrates for that enzyme. Similarly, one may anticipate that not all pyridinium metabolites of MPTP analogs would be efficient substrates for the dopamine uptake carrier. The separate and distinct structural requirements for these first two steps in MPTP neurotoxicity may result in retention of neurotoxicity by relatively few structural analogs of MPTP, and previous publications (7,8) have indeed indicated that several close congeners of MPTP are devoid of measurable neurotoxic liability. Our findings appear to be the first showing that a heterocyclic replacement of the phenyl group on MPTP results in some retention of neurotoxic potential.

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