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Prevention of dopaminergic toxicity of MPTP in mice by phenylethylamine, a specific substrate of type B monoamine oxidase

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N-methyl-4-phenyl-1, 2, 5, 6-tetrahydropyridine (MPTP) is toxic to dopaminergic neurones in several mammalian species including mice. Combined treatment with phenylethylamine prevented in mice the long-term (30 days post-treatment) dopamine depletions in striatum induced by MPTP. Phenylethylamine, a naturally-occuring specific substrate of monoamine oxidase (MAO) type B, probably protects against effects of MPTP by competitively inhibiting the oxidative conversion of MPTP to its toxic metabolite N-methyl-4-phenylpyridinium ion catalysed by MAO-B.

Introduction N-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) is toxic to dopaminergic neurones in several mammalian species including mice (Heikkilla *et al.*, 1984a; Hallman *et al.*, 1985). Several studies suggest that the enzyme monoamine oxidase (MAO) type B which is specific for dopamine degradation, has an essential role in the mechanism of action of MPTP probably by catalysing its oxidative conversion to the toxic metabolite N-methyl-4-phenylpyridinium ion (MPP^+) (Markey *et al.*, 1984). Oxidation of MPTP to MPP^+ by rat brain mitochondrial fractions is blocked by coincubation with pargyline and deprenyl but not with clorgyline, selective inhibitors of MAO-B and MAO-A, respectively (Chiba *et al.*, 1984; Parsons & Rainbow, 1984). MPTP is a potent *in vitro* inhibitor of MAO (Chiba *et al.*, 1984; Parsons & Rainbow, 1984). Distribution of [^{3}H]-MPTP binding sites overlap with those of [^{3}H]-pargyline in rat brain suggesting that it corresponds to the anatomical location of MAO activity (Parsons & Rainbow, 1984; Rainbow *et al.*, 1985). In addition, damage by MPTP to dopaminergic neurones is prevented in mice (Heikkilla *et al.*, 1984b) and monkeys (Langston *et al.*, 1984) *in vivo*, and in explants of rat embryo mesencephalon *in vitro* (Mytilineou & Cohen, 1984) by cotreatment with MAO-B inhibitors. We have now shown that systemic administration of phenylethylamine, a naturally occurring specific substrate for MAO-B (Yang & Neff, 1973; McQuade, 1984) prevents in mice the long-term MPTP-induced dopamine depletions in striatum.

Methods C57 black mice (initial weight 25–35 g) were injected subcutaneously with MPTP (Aldrich, 30 mg kg^{-1} dissolved in 30% ethanol) or with vehicle, once daily for two consecutive days. A group of MPTP-treated animals were injected intraperitoneally with phenylethylamine (50 mg kg^{-1}) once daily, for six consecutive days, two days before, two days in combination with, and two days after MPTP injections. An additional group was given phenylethylamine alone for six days. Mice were decapitated 30 days after the last injection, brains were rapidly removed and striata dissected, frozen on dry ice and homogenized in 0.1M perchloric acid. Deproteinized aliquots were extracted with dihydroxybenzylamine as an internal standard on miniature columns containing activated alumina and assayed for dopamine by high-performance liquid chromatography with electrochemical detection (Hefti *et al.*, 1981).

Results Injections of MPTP alone produced marked depletions (by about 50%) in striatal dopamine concentrations at 30 days post-treatment indicating persistent damage to nigrostriatal dopaminergic nerve-endings (Table 1). Administration of phenylethylamine alone, once daily for six days, did not affect dopamine levels in striatum at 30 days after the last injection. Combined treatment with MPTP and phenylethylamine completely prevented the fall in striatal dopamine content induced by MPTP. Dopamine levels in animals given MPTP and phenylethylamine were similar to those in controls and in phenylethylamine-treated mice at 30 days post-treatment (Table 1).

Discussion The present study shows that phenylethylamine administered systemically prevents the long-term MPTP-induced striatal dopamine depletions indicating that it can protect against damaging effects of the neurotoxin. Phenylethylamine protection is probably not mediated via its enhancing effect on dopamine synthesis and release (McQuade & Wood,

Table 1 Effect of combined systemic administration of phenylethylamine on long-term N-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP)-induced decrease in dopamine concentrations in mouse striatum

	n	*Dopamine* (ng mg^{-1} protein)
Controls	8	144 ± 6
MPTP	10	74 ± 1*
Phenylethylamine	7	139 ± 6
MPTP + phenylethylamine	9	134 ± 8

Means ± s.e.mean; MPTP was injected once daily for two consecutive days (30 mg kg^{-1}, s.c.) alone or in combination with phenylethylamine given once daily for six days (50 mg kg^{-1}, i.p.), two days before, two days in conjunction with and two days after MPTP. An additional group received phenylethylamine alone once daily for six days. Mice were decapitated 30 days after last injection. *Significantly lower than in other groups ($P < 0.001$; t test).

1983) or its possible direct stimulant action on postsynaptic dopamine receptors (Antelman *et al.*, 1977). We have shown that acceleration of dopamine turnover or stimulation of dopamine receptors by combined treatment with haloperidol or the direct dopamine agonists, apomorphine and bromocriptine, respectively, had no effect on dopamine toxicity of MPTP in mice (Melamed *et al.*, 1985). We have also demonstrated that cotreatment with the dopamine uptake inhibitor, nomifensine, prevents toxicity of MPTP by blocking toxin transport into dopaminergic nerve-terminals via the specific dopamine reuptake system (Melamed *et al.*, 1985). It is unlikely that phenylethylamine protection is due to a similar mechanism since it is only a weak inhibitor of dopamine uptake (Raiteri *et al.*, 1978).

The specific MAO-B inhibitors, pargyline and deprenyl, protect dopaminergic neurones against MPTP toxicity (Heikkilla *et al.*, 1984b; Langston *et al.*, 1984; Mytilineou & Cohen, 1984) by binding covalently and irreversibly to the flavin adenine dinucleotide at the active centre of the enzyme (Sallah *et al.*, 1979). Phenylethylamine is a specific substrate for MAO-B (Yang & Neff, 1973; McQuade, 1984) and its affinity for the enzyme is greater than those of dopamine and MPTP (Youdim *et al.*, 1985). It is therefore feasible that phenylethylamine prevents MPTP-induced dopamine depletions by competitively inhibiting the oxidative conversion of MPTP to MPP^+ catalysed by MAO-B. Rats are relatively resistant while monkeys and humans are highly susceptible to the toxic effects of MPTP (Boyce *et al.*, 1984; Chiueh *et al.*, 1984). The causes for the species differences in dopamine toxicity of MPTP are largely undetermined. Phenylethylamine is a naturally occurring substance in mammalian brain and it has been suggested that its concentrations are higher in rat than in human central nervous system (McQuade, 1984). This raises an interesting possibility that immunity of rats to MPTP may be due, in part, to greater endogenous levels of phenylethylamine and perhaps of other specific substrates for MAO-B, which may inhibit competitively the MAO-B dependent oxidative metabolism of MPTP to MPP^+.

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