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A high dose of MPTP overcomes the protective effect of selegiline against dopaminergic neurotoxicity

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Abstract—1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) at 90 mg kg^{-1} s.c., a dose lethal in non-pretreated mice, was well tolerated in selegiline ((−)-deprenyl)-pretreated mice and produced persistent depletion of striatal dopamine and its metabolites one week after the last of four daily injections. The protective effect of selegiline against dopaminergic neurotoxicity of MPTP can thus be overridden by a high dose of MPTP that would be lethal without selegiline pretreatment.

1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) was discovered to cause parkinsonian symptoms in human drug abusers who inadvertently self-administered it (Langston et al 1983) and then was shown to cause selective neurotoxicity to nigrostriatal dopamine neurons, accompanied by severe motor dysfunction, in non-human primates (Burns et al 1983; Langston et al 1984). In mice, MPTP causes persistent depletion of striatal dopamine and its metabolites (Heikkila et al 1984a), loss of dopamine uptake capacity in the striatum (Wallace et al 1984; Sershen et al 1984), and histologic evidence of cell loss in the substantia nigra (Gupta et al 1984). The neurotoxic effect of MPTP toward nigrostriatal dopamine neurons requires metabolic conversion of MPTP to 1-methyl-4-phenyl-pyridinium (MPP+) via monoamine oxidase type B (MAO-B) (Chiba et al 1984), and selegiline ((−)-deprenyl) and other inhibitors of MAO-B prevent the neurotoxicity in primates (Langston et al 1984) and in mice (Heikkila et al 1984b).

Recently we found that the acute lethality of MPTP in mice is also prevented by inhibitors of MAO-B (Fuller et al 1988). In selegiline-pretreated mice, high doses of MPTP that would otherwise be lethal could be given without lethality or overt signs of toxicity. The study described here was done to see if a high dose of MPTP could override the protective effect of selegiline against striatal neurotoxicity. The results show that a 90 mg kg^{-1} dose of MPTP, which is lethal ordinarily, is well tolerated in selegiline-pretreated mice and produces persistent depletion of striatal dopamine and its metabolites analogous to that seen with lower doses of MPTP in non-pretreated mice.

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Male CRL/CFW mice, 20–30 g, were purchased from Charles River Breeding Laboratories (Portage, MI). MPTP hydrochloride was synthesized by Dr David W. Robertson in the Lilly Research Laboratories, and selegiline was a gift from Professor J. Knoll, Semmelweis School of Medicine, Budapest, Hungary. Mice were killed by cervical dislocation. Brains were removed and dissected, then striata were frozen on dry ice and stored at −60°C. 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).

Table 1 gives the concentrations of dopamine and its metabolites in mouse striatum after several treatment paradigms. MPTP at 30 mg kg^{-1} caused marked depletion of striatal dopamine and slightly less depletion of DOPAC and HVA. Four doses of selegiline, before each of the four MPTP doses, completely prevented the depletion of dopamine and its metabolites. A higher dose of MPTP, 90 mg kg^{-1}, which is ordinarily lethal (Fuller et al 1988) but which could be given to selegiline (4)-pretreated mice without any lethality or severe effects that could be observed, caused significant depletion of dopamine and its metabolites, though less than that produced by a 30 mg kg^{-1} dose of MPTP given without selegiline. When the 90 mg kg^{-1} dose of MPTP was given to mice pretreated with only one dose of selegiline (1 h before the first dose of MPTP), it also was well tolerated by the mice and caused more marked depletion of dopamine and its metabolites, approaching the magnitude of the depletion seen with the 30 mg kg^{-1} dose of MPTP given alone. Neither of the selegiline treatment regimens alone caused any effects on dopamine or its metabolites one week later.

The current findings show that a high dose of MPTP, which would be lethal in non-pretreated mice but which is well tolerated in selegiline-pretreated mice, can override the protective effects of selegiline against selective neurotoxicity toward nigrostriatal dopamine neurons. Because selegiline is an irreversible inhibitor of MAO-B causing inhibition of that enzyme for as long as 14 days (Fuller et al 1988), a single dose of selegiline

Table 1. Effect of MPTP on striatal dopamine and its metabolites in control mice and in selegiline-pretreated mice.

	Concentrations in striatum, nmol g^{-1}		
Treatment group	Dopamine	DOPAC	HVA
Control	86·5 ± 3·4	5·48 ± 0·10	5·95 ± 0·20
MPTP (30 mg kg^{-1})	19·8 ± 2·2* (−77%)	1·80 ± 0·22* (−67%)	2·94 ± 0·21* (−51%)
MPTP (30 mg kg^{-1}) + selegiline (4)	86·1 ± 3·9	6·31 ± 0·42	5·99 ± 0·27
MPTP (90 mg kg^{-1}) + selegiline (4)	57·2 ± 7·6* (−34%)	3·99 ± 0·44* (−27%)	4·38 ± 0·35* (−26%)
MPTP (90 mg kg^{-1}) + selegiline (1)	33·2 ± 2·8* (−62%)	2·64 ± 0·28* (−52%)	3·30 ± 0·17* (−45%)
Selegiline (4)	85·4 ± 4·4	5·13 ± 0·39	5·65 ± 0·36
Selegiline (1)	83·7 ± 4·4	5·62 ± 0·22	5·39 ± 0·14

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

Daily s.c. injections of MPTP hydrochloride (30 or 90 mg kg^{-1}) were given for 4 days, and mice were killed one week after the last injection. Some mice were pretreated with selegiline (10 mg kg^{-1} i.p.) given 1 h before the first injection of MPTP [selegiline (1)] or with selegiline (1 mg kg^{-1} i.p.) given 1 h before each of the 4 injections of MPTP [selegiline (4)]. Mean values ± standard errors for 5–10 mice per group are shown.

has earlier been shown to prevent the persistent depletion of striatal dopamine caused by four daily 20 mg kg^{-1} doses of MPTP (Fuller et al 1987). Four daily 90 mg kg^{-1} doses of MPTP, not tolerated in non-pretreated mice, could be given to mice after a single dose of selegiline and caused marked depletion of striatal dopamine, DOPAC and HVA one week later. Even four daily doses of selegiline—one before each dose of MPTP—failed to protect completely against the persistent effects of this high dose of MPTP, although they did prevent the persistent effects of a lower (30 mg kg^{-1}) dose of MPTP. We had earlier reported that the 10 mg kg^{-1} i.p. dose of selegiline inhibited MAO-B nearly completely (92%) at 1 h in mouse brain but did not inhibit MAO-A at all (Fuller & Hemrick-Luecke 1984). By 4 days, MAO-B activity had recovered partially but was still inhibited by 67%. Thus in the two groups receiving MPTP at 90 mg kg^{-1} in Table 1, enough remaining MAO-B activity may have existed to form the amounts of MPP+ reported by Fuller et al (1988). Alternatively, some MPP+ may have been formed by MAO-A after the high doses of MPTP. In either case, we had earlier found that MPP+ concentrations in brain after a 90 mg kg^{-1} dose of MPTP were nearly as high in selegiline-pretreated mice as were MPP+ concentrations in brain after a 30 mg kg^{-1} dose of MPTP in non-pretreated mice (Fuller et al 1988). Thus the ability of the high dose of MPTP to override the protective effect of selegiline against selective neurotoxicity is consistent with extensive evidence implicating MPP+ in that neurotoxicity (Chiba et al 1984; Heikkila et al 1984b).

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