Effects of Noradrenergic Lesions on MPTP/MPP⁺ Kinetics and MPTP-Induced Nigrostriatal Dopamine Depletions

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ABSTRACT

Norepinephrine (NE) depletion caused by damage to locus ceruleus neurons was shown to worsen experimental Parkinsonism induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in monkeys and in rodents. However, it is not clear whether the lesion to the NE system enhances neurotoxicity in the nigrostriatal dopaminergic (DA) pathway and/or impairs the recovery of DA neurons once the neurotoxic insult is generated. In this study, we provide evidence that the lesion of NE terminals, induced by the selective neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4; 50 mg/kg), must occur before MPTP (30 mg/kg) administration in order to enhance MPTP toxicity. As a second step, we evaluated the acute effects of MPTP on the nigrostriatal DA pathway in NE-lesioned animals compared with intact animals. We observed a more marked acute DA depletion, persisting at 12 h, in DSP-4 + MPTP-treated mice compared with MPTP-injected controls. These findings, combined with the lack of an MPTP enhancement when NE depletion was induced 12 h after MPTP administration, suggest that in NE-depleted animals, a more pronounced acute neuronal sensitivity to MPTP occurs. In line with the hypothesis of an acute protective effect by NE axons, we evaluated whether the enhancement of MPTP toxicity in NE-lesioned animals is achieved through alterations to the kinetics of MPTP and its metabolite. Our findings indicate that despite the pivotal role of NE terminals in taking up and storing 1-methyl-4-phenylpyridinium (MPP⁺), MPTP enhancement does not depend on modifications in the striatal kinetics of MPTP/MPP⁺ measured at seven different time intervals after MPTP administration.

Impairment of the central NE system in neurodegenerative disorders, and particularly in Parkinson's disease, has been well documented by several neuropathological findings (Alvord and Forno, 1992). Biochemical studies performed post mortem confirmed the coexistence of a massive NE depletion along with DA loss in parkinsonian patients (Hornykiewicz and Kish, 1987). Although the significance of this alteration remains unknown, recent evidence suggests a causative role for NE depletion in worsening the neurotoxic damage to the nigrostriatal DA pathway (Mavridis et al., 1991; Marien et al., 1993; Bing et al., 1994; Fornai et al., 1995b; Fornai et al., 1996).

Particularly, in the first study performed in primates, NE depletion caused by damage to LC neurons was shown to worsen experimental parkinsonism induced by the neurotoxin MPTP (Mavridis et al., 1991). These findings were later confirmed by studies performed in rodents (Bing et al., 1994; Marien et al., 1993). These data were obtained either by lesioning the NE perikarya with a focal injection (within the LC) of 6-OH-DA (Mavridis et al., 1991; Bing et al., 1994) or by damaging NE axons via systemic administration of DSP-4 (Marien et al., 1993). Further studies extended the protective role of NE terminals originating from the LC to other models of nigrostriatal damage (Fornai et al., 1995b; 1996).

Despite the evidence provided in these previous studies, it is not clear whether the lesion of the NE system enhanced neurotoxicity in the nigrostriatal DA pathway and/or impaired the recovery of DA neurons once the neurotoxic insult was generated. In their study performed in primates, Mavridis et al. (1991) suggested, on the basis of behavioral data, that the deleterious effects of an LC lesion might be the consequence of an impaired recovery of nigrostriatal DA neurons of primates treated 3 months before with MPTP. Subsequently, Marien et al. (1993) showed that LC-lesioned animals had a lower DA content than controls as early as 7 days after the administration of MPTP. These latter findings suggest an enhancement of MPTP toxicity rather than an impairment of recovery once the lesion was produced.

However, this issue should be addressed further by investigating whether, to worsen experimental parkinsonism, the lesion of NE terminals needs to occur before MPTP administration or whether it can be produced immediately after

ABBREVIATIONS: DA, dopamine; DSP-4, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; LC, locus ceruleus; MPP⁺, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NE, norepinephrine; SN, substantia nigra.
MPTP injection. Again, following the hypothesis of increased toxicity, the acute effects of MPTP on the nigrostriatal DA pathway in LC-lesioned compared with intact animals should be investigated. In line with the hypothesis of an acute protective effect by NE axons, it should be determined whether the enhancement of MPTP toxicity in NE-lesioned animals is achieved through alterations to the kinetics of MPTP and its metabolite. This point might be particularly crucial because, as shown by Herkenham et al. (1991), NE varicosities represent a central site that avidly takes up and densely retains MPP\(^+\) after systemic injections of MPTP. Indeed, LC axons have shown a great capacity for storing MPP\(^+\)—even higher than that of the SN pars compacta neurons (Herkenham et al., 1991). In this way, the NE system might play a buffering role by reducing the availability of MPP\(^+\) to DA neurons. Conversely, the selective lesion of NE axons arising from the LC might increase the availability of MPP\(^+\) (fig. 1). Additionally, the extraneuronal (glial) transport mechanism for NE (uptake 2) avidly transports MPP\(^+\) (Russe et al., 1992); as a consequence, the absence of endogenous NE might be crucial in increasing MPP\(^+\) uptake. Finally, it is well known that the lesion of NE axons produces general effects on body temperature, basal metabolism and cardiovascular activity. All these effects might influence either the delivery of MPTP to the striatum or the metabolic rate of its conversion to MPP\(^+\) within the basal ganglia. If at least some of these mechanisms were responsible for the DSP-4-induced enhancement of MPTP toxicity, we would expect significant variations in the striatal kinetics of MPTP/MPP\(^+\) in LC-lesioned compared with intact animals.

To address all these critical issues, in this study we pretreated C57Bl/6J mice with the NE neurotoxin DSP-4, which induces a selective pattern of NE loss that involves NE terminals arising from the LC (Jonsson et al., 1981; Grzanna et al., 1989). We then administered MPTP at a dose that, by itself, moderately reduced striatal DA levels. Mice were administered MPTP either 12 h before or 3 days after DSP-4. In these animals, we studied both the DSP-4-induced enhancement of MPTP-induced striatal DA depletion at different time intervals and the kinetics of MPTP and MPP\(^+\) in several brain areas after the same treatment. A preliminary report of some of the results (striatal MPP\(^+\) kinetics) has been presented (Fornai et al., 1995c).

**Materials and Methods**

**Animals.** Male C57/6N Black mice (Charles River, Calco, Como, Italy), 8 to 9 weeks old and weighing 20 to 24 g, were kept under environmentally controlled conditions (12 h light/dark cycle with light on between 07.00 and 19.00; room temperature 21°C) with food and water ad libitum. Animals were treated in accordance with the Guidelines for Animal Care and Use of the National Institutes of Health. The experiments described in this article were approved by the School of Medicine Ethical Committee on Experimental Studies at the University of Pisa.

**Experimental design.** In the first set of experiments, mice were treated i.p. with a single dose of DSP-4 hydrochloride (Sigma Chemical Co., Saint Louis, MO; 50 mg/kg). Then, 3 days after DSP-4, a single dose of MPTP (Research Biochemicals Inc. (RBI), Natick, MA) was administered i.p. The dose of MPTP (30 mg/kg) was selected to produce an intermediate degree of striatal DA loss (Corsini et al., 1985).

Control groups received saline, MPTP or DSP-4 alone, at the same doses and times used for the group given the combined treatment. Three days after the DSP-4 injection, a group of animals treated with DSP-4, together with a group of saline-injected controls, were sacrificed to assay regional cerebral monoamine levels at the time of MPTP administration. Seven days after MPTP injection (10 days after DSP-4), the remaining animals were killed.

The second set of experiments was performed to investigate whether the exacerbation of MPTP-induced parkinsonism in LC-lesioned animals observed at 7 days after MPTP might result from an impaired recovery of striatal DA levels during this time interval. This experiment was carried out by injecting DSP-4 12 h after MPTP administration, when MPP\(^+\) was completely cleared from the striatum (see “Results”). Animals were sacrificed 7 days after MPTP administration (61⁄2 days after DSP-4).

Another series of experiments was performed immediately after MPTP administration to evaluate the effects of DSP-4-induced NE depletion on the kinetics of striatal MPTP/MPP\(^+\) and on MPTP-induced acute striatal DA depletion as well. Animals were treated i.p. with a single dose of either DSP-4 hydrochloride (50 mg/kg, Sigma) or saline. Three days later, all animals but controls were administered a single i.p. injection of MPTP (RBI, 30 mg/kg). Ani-
imals were sacrificed at seven time intervals (10 min, 30 min, and 1, 2, 4, 6 and 12 h) after MPTP to evaluate the kinetics of MPTP (entry, storage and clearance) and MPP⁺ (formation, storage and clearance) within the different brain regions. The same animals were studied to evaluate, at seven time intervals, the acute effects of MPTP on striatal DA depletion in untreated mice and in DSP-4 pretreated animals.

In each series of experiments, mice were killed by cervical dislocation, and the brains were immediately removed. The frontal cortex, the hypothalamus, the cerebellum, the SN and the striatum were dissected and assayed for NE, DA, serotonin (5HT) and metabolites. In particular, the frontal cortex was dissected at the level of the frontal pole, and the dorsal striatum was dissected using the external walls of the lateral ventricles as the internal limits and the corpus callosum as the external boundary. The hypothalamus was dissected from the ventral surface of the brain and separated ventrally from the hypophysis and dorsally from the thalamus. The cerebellum was dissected at the level of the posterior vermis. The dorsal hippocampus was isolated internally from a surface constituted ventrally by the entorhinal cortex and the amygdala complex and dorsally by the parietal cortex, and it was removed at the level of the cerebral peduncles. At the same level, the SN was dissected from the ventral mesencephalon using a magnifying lens.

**Assay of catecholamines.** The striatum, the hypothalamus, the frontal cortex, the hippocampus and the cerebellum were sonicated in 0.6 ml, and the SN in 0.2 ml, of ice-cold 0.1 M perchloric acid containing 10 ng/ml of 3,4-dihydroxybenzylamine (DBA, Sigma) as internal standard. An aliquot of the homogenate (50 μl) was assayed for protein (Lowry et al., 1951). After centrifugation at 8000 × g for 10 min, 20 μl of the clear supernatant was injected into an HPLC system where NE, DA, 5HT and metabolites were analyzed as previously described (Fornai et al., 1995b).

**Assay of MPTP and MPP⁺ levels.** The striatum, the frontal cortex, the hippocampus, the hypothalamus and the cerebellum of mice and rats were sonicated in 0.5 ml, and the SN in 0.2 ml, of ice-cold 0.1 M perchloric acid containing a known amount (500 ng/ml) of MPTP-3-ole used as the internal standard. An aliquot of the homogenate (50 μl) was assayed for protein (Lowry et al., 1951). After centrifugation, the clear supernatant was injected into an HPLC coupled with a UV detector to analyze MPP⁺ and MPTP as previously described (Miele et al., 1995), with minor modifications. Briefly, the UV detector was programmed to change the wavelength automatically: for MPTP-3-ole the wavelength was 260 nm, for MPTP 245 nm and for MPP⁺ 295 nm.

**Data analysis.** For catecholamine assays, a standard curve was prepared using known amounts of DA, NE and metabolites (Sigma) dissolved in 0.1 M perchloric acid containing a constant amount (10 pg/μl) of the internal standard (DBA), as used for tissue samples. The standard curve for each compound (DA, NE or its metabolite) was calculated using regression analysis of the ratios of the peak areas (compound area/DBA area) for various concentrations of each compound recorded at the reducing electrode. An analogous regression analysis was performed for the oxidizing electrode. For the MPTP and MPP⁺ assay, a standard curve was prepared using known amounts of the compounds (RBI) dissolved in 0.1 M perchloric acid containing 500 ng/ml of MPTP-3-ole as the internal standard. The ratios of the peak areas for either MPP⁺/MPTP-3-ole or MPTP/ MPTP-3-ole were measured, and regression curves were determined using various concentrations of MPTP and MPP⁺. For NE and DA levels, results are expressed as the mean ± S.E.M. of 12 animals per group. For MPTP, MPP⁺ and DA levels measured acutely after MPTP administration, results are expressed as the mean ± S.E.M. of 10 animals per group. The effects of MPTP and of the various combined treatments on striatal catecholamine levels, as well as on MPTP/MPP⁺ striatal levels, were statistically evaluated using analysis of variance with Sheffe’s post-hoc analysis. The null hypothesis was rejected when P < .05.

**Results**

**DSP-4 Induces a Specific Pattern of NE Depletion**

Three days after administration, DSP-4 (50 mg/kg i.p.) induced a pattern of NE loss following or reproducing the trim of innervation of LC axon terminals (table 1). In particular, the hippocampus and the cerebellar cortex were most affected with a massive NE loss, whereas the frontal cortex was markedly depleted (80%) and the SN displayed a moderate degree (~50%) of NE decrease. By contrast, neither the hypothalamus nor the striatum showed significant changes in NE levels after DSP-4 administration. Dopamine levels were unaffected by DSP-4 in all the brain areas under examination (table 1).

**Chronic NE Depletion Enhances MPTP-Induced Chronic Striatal DA Loss**

As shown in figure 1A, MPTP (30 mg/kg i.p.) produced an intermediate degree of striatal DA loss 7 days after treatment. In animals administered DSP-4 (10 days before assay) and then given MPTP (7 days before assay) there was a significant enhancement of MPTP-induced striatal DA depletion. By contrast, as found 3 days after DSP-4 administration, there was no change 10 days after treatment in striatal DA levels of mice treated only with DSP-4. Similar data were obtained for nigral DA levels (fig. 1 B).

**NE Depletion Induced 12 h After MPTP Administration Does Not Modify Striatal DA Loss**

Injection of DSP-4 (50 mg/kg) when MPP⁺ was completely cleared from the striatum, 12 h after MPTP (30 mg/kg), resulted in an intermediate degree of striatal DA depletion 7 days after MPTP administration, compared with controls. Despite the marked effects of DSP-4 on cerebral NE levels (table 2), the degree of striatal DA loss did not differ from that observed in animals injected with MPTP alone (table 2)

**TABLE 1**

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Saline</th>
<th>DSP-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Values are given in ng/mg of protein)</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>5.09 ± 0.34</td>
<td>0.22 ± 0.04*</td>
</tr>
<tr>
<td>DA</td>
<td>0.73 ± 0.08</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>0.20 ± 0.02</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>DA</td>
<td>125.16 ± 3.85</td>
<td>132.86 ± 4.08</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>4.28 ± 0.14</td>
<td>0.53 ± 0.02*</td>
</tr>
<tr>
<td>DA</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>4.51 ± 0.37</td>
<td>1.05 ± 0.07*</td>
</tr>
<tr>
<td>DA</td>
<td>1.04 ± 0.06</td>
<td>0.95 ± 0.07</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>15.17 ± 0.73</td>
<td>13.84 ± 0.09</td>
</tr>
<tr>
<td>DA</td>
<td>3.29 ± 0.30</td>
<td>3.62 ± 0.34</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>11.24 ± 0.68</td>
<td>5.98 ± 0.31*</td>
</tr>
<tr>
<td>DA</td>
<td>5.75 ± 0.19</td>
<td>7.03 ± 0.88</td>
</tr>
</tbody>
</table>

* P < .05 compared with controls.
Time course of MPTP-induced acute striatal DA loss. MPTP and MPP⁺ levels in six different brain regions at seven different time intervals after MPTP (30 mg/kg). Three days before MPTP injection, mice were administered either saline or DSP-4 (50 mg/kg) to produce a selective lesion of NE terminals arising from the LC. Results are obtained from 10 animals per group sacrificed at each time interval and are expressed as the mean ± S.E.M. Differences among groups were evaluated using ANOVA with Sheffe’s post-hoc analysis. *P < .05 compared with controls. **P < .01 compared with controls.

Fig. 2. Time course of MPTP-induced acute striatal DA loss. MPTP and MPP⁺ levels in six different brain regions at seven different time intervals after MPTP (30 mg/kg). Three days before MPTP injection, mice were administered either saline or DSP-4 (50 mg/kg) to produce a selective lesion of NE terminals arising from the LC. Results are obtained from 10 animals per group sacrificed at each time interval and are expressed as the mean ± S.E.M. Differences among groups were evaluated using ANOVA with Sheffe’s post-hoc analysis. *P < .05 compared with controls. **P < .01 compared with controls.

Effects of NE Loss on the Time Course of Acute MPTP-Induced Striatal DA Depletion

As shown in figure 2, immediately after MPTP administration (10, 30 and 60 min after injection) no differences in DA levels were observed in mice treated with MPTP or DSP-4 + MPTP compared with saline-injected controls (fig. 2). At 2 h and 4 h after MPTP administration, a progressive decline of striatal DA levels occurred in MPTP-treated animals, and there was a significantly more pronounced effect in DSP-4 + MPTP-treated mice. At 6 h and 12 h after MPTP administration, striatal DA levels remained reduced in MPTP-treated mice, and the reduction was constantly and significantly more pronounced in DSP-4 + MPTP-treated animals.

Effects of the Loss of Noradrenergic Terminals on the MPTP/MPP⁺ Kinetics in Different Brain Regions

Striatum. As shown in table 3, no difference was measured in MPTP levels 10 min after MPTP administration within the striata of saline + MPTP- and DSP-4 + MPTP-injected animals. In both groups, striatal MPTP completely disappeared 30 min after MPTP administration and was converted to similar amounts of striatal MPP⁺. Striatal MPP⁺ was virtually absent at 10 min (only one animal in each group showed a minimal amount of striatal MPP⁺ at 10 min) and clearly appeared at 30 min after MPTP administration. At 60 min there was a slight increase in striatal MPP⁺ levels of animals treated with saline + MPTP compared with DSP-4 + MPTP. By contrast, 2 h after MPTP administration, striatal MPP⁺ levels were higher in DSP-4 + MPTP-treated animals compared with mice given saline + MPTP. Four h after treatment, striatal MPP⁺ levels were higher in mice given MPTP alone, and a similar difference was observed at 6 h. At 12 h, there were minimal but detectable amounts of striatal MPP⁺ only in this group of animals.

SN. No difference in MPTP levels within the SN was observed between the two groups of animals 10 min after systemic MPTP administration (table 3). A marked difference between the two groups was observed at 30 min, at which time we observed a full persistence of nigral MPTP in mice treated with DSP-4 + MPTP and a significant decrease in animals administered saline + MPTP. This difference was even more pronounced at 60 min. An opposite trend was observed for the early time course of nigral MPP⁺. At 30 min, there was a pronounced increase in nigral MPP⁺ in animals administered saline + MPTP compared with animals treated with DSP-4 + MPTP. This difference was still present 60 min after MPTP administration, whereas at 2 h, the two groups of animals had similar amounts of striatal MPP⁺. By contrast,
at 4 h, nigral MPP⁺ levels were higher in DSP-4 + MPTP-injected animals than in mice given saline + MPTP, and the same difference was detected at 6 h. Twelve hours after MPTP injection, no detectable amounts of nigral MPP⁺ were found in either experimental group (table 3).

**Hypothalamus.** No difference in MPTP levels was observed at 10 min between DSP-4 + MPTP-treated animals and mice given MPTP alone (table 3). Hypothalamic MPTP was markedly, though to a similar extent, reduced in both groups at 30 min, and it was undetectable in either group of animals at 60 min. At 10 and 30 min, MPP⁺ levels progressively increased, although no difference was observed between the two groups of animals. At 60 min, MPP⁺ levels remained stable in both groups, whereas similar decreases in hypothalamic MPP⁺ were detected at 2 h. At 4 h, there was a slight but significant prevalence of MPP⁺ in saline + MPTP-treated animals compared with mice given DSP-4 + MPTP. The same was true 6 h after MPTP administration. No hypothalamic MPP⁺ was detected in either group 12 h after MPTP administration.

**Frontal cortex.** No differences in frontocortical MPTP entry were measured between the two treatments 10 min after MPTP administration, and these values corresponded to the highest amounts of MPTP measured in the different brain areas under examination (table 4). By contrast, very small levels of MPP⁺ were measured at 10 min. From 30 min on, MPTP was no longer detectable in animals treated with saline + MPTP, whereas considerable MPTP levels were present in DSP-4 + MPTP-treated mice, some of which still had detectable levels at 60 min. At 30 min, MPP⁺ levels were

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### Table 3
**MPTP/MPP⁺ kinetics in striatum, SN and hypothalamus at seven different time intervals**
MPTP and MPP⁺ levels at seven different time intervals after the administration of MPTP (30 mg/kg). Three days before MPTP administration, mice were injected with either saline or DSP-4 (50 mg/kg) to produce a selective lesion of NE terminals arising from the LC. Results are obtained from 10 animals sacrificed at each time interval from each group and are expressed as the mean ± S.E.M. Differences among groups were evaluated using ANOVA with Sheffé’s test.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Striatum</th>
<th>SN</th>
<th>Hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPTP</td>
<td>DSP-4 + MPTP</td>
<td>MPTP</td>
</tr>
<tr>
<td>10 min</td>
<td>109.1 ± 9.1</td>
<td>107.7 ± 8.2</td>
<td>45.5 ± 7.5</td>
</tr>
<tr>
<td>30 min</td>
<td>0.2 ± 0.2</td>
<td>1.3 ± 1.3</td>
<td>21.8 ± 3.6</td>
</tr>
<tr>
<td>1 h</td>
<td>/</td>
<td>/</td>
<td>20.9 ± 6.5</td>
</tr>
<tr>
<td>2 h</td>
<td>46.3 ± 4.3</td>
<td>44.2 ± 4.0</td>
<td>80.9 ± 12.8</td>
</tr>
</tbody>
</table>

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### Table 4
**MPTP/MPP⁺ kinetics in frontal cortex, hippocampus, and cerebellum at seven different time intervals**
MPTP and MPP⁺ levels at seven different time intervals after the administration of MPTP (30 mg/kg). Three days before MPTP administration, mice were injected with either saline or DSP-4 (50 mg/kg) to produce a selective lesion of NE terminals arising from the LC. Results are obtained from 10 animals sacrificed at each time interval from each group and are expressed as the mean ± S.E.M. Differences among groups were evaluated using ANOVA with Sheffé’s test.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Frontal Cortex</th>
<th>Hippocampus</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPTP</td>
<td>DSP-4 + MPTP</td>
<td>MPTP</td>
</tr>
<tr>
<td>10 min</td>
<td>206.2 ± 13.9</td>
<td>228.4 ± 17.2</td>
<td>138.9 ± 11.4</td>
</tr>
<tr>
<td>30 min</td>
<td>3.2 ± 0.4</td>
<td>3.5 ± 0.1</td>
<td>23.3 ± 2.6</td>
</tr>
<tr>
<td>1 h</td>
<td>/</td>
<td>68.1 ± 5.2*</td>
<td>/</td>
</tr>
<tr>
<td>2 h</td>
<td>42.2 ± 4.9</td>
<td>14.7 ± 2.6*</td>
<td>37.6 ± 2.9</td>
</tr>
<tr>
<td>4 h</td>
<td>76.2 ± 6.3</td>
<td>140.6 ± 11.7*</td>
<td>38.4 ± 3.5</td>
</tr>
<tr>
<td>6 h</td>
<td>23.2 ± 3.4</td>
<td>57.8 ± 3.7*</td>
<td>/</td>
</tr>
<tr>
<td>12 h</td>
<td>/</td>
<td>55.2 ± 4.6*</td>
<td>/</td>
</tr>
</tbody>
</table>

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* P < .05 compared with MPTP.
significantly higher in saline + MPTP-treated than in DSP-4 + MPTP-treated mice, and a similar difference was detected at 60 min. At 2 h, by contrast, MPP⁺ levels of DSP-4 + MPTP-treated mice were twice the levels of animals given saline + MPTP, and the same difference was measured at 4 h, whereas at 6 h, MPP⁺ was detectable only in animals given DSP-4 + MPTP. At 12 h, MPP⁺ was absent from both groups of animals (table 4).

**Hippocampus.** As shown in table 4, there was a MPTP persisted longer in the hippocampus of NE-depleted animals compared with controls. In particular, although MPTP levels were similar at 10 min, no amounts of hippocampal MPTP were detectable 30 min in any of the saline + MPTP-treated animals, whereas there was still MPTP in DSP-4 + MPTP-treated mice. At 60 min, MPTP was absent in both groups of animals. No difference in MPP⁺ levels was measured 10 min after MPTP administration. Thirty minutes after injection, MPP⁺ was more concentrated in saline + MPTP-treated animals, whereas at 60 min and at 2 h, the amount of MPP⁺ was similar in both groups of animals. By contrast, 4 h and 6 h after treatment, MPP⁺ was absent in saline + MPTP-treated mice, whereas it was still detectable at 4 h and 6 h in mice given DSP-4 + MPTP. Twelve hours after treatment, MPP⁺ was cleared from the hippocampus of both groups of animals (table 4).

**Cerebellum.** Similar cerebellar amounts of MPTP were detected at 10 min in the two groups, whereas at 30 min, MPTP was no longer detectable in saline + MPTP-treated animals, though it persisted in DSP-4 + MPTP-treated mice (table 4). At 60 min, MPTP disappeared from the cerebellar cortex of both groups of animals. Small amounts of MPP⁺ were measured in both groups 10 min after treatment, and similar amounts were also detected at 30 min. By contrast, at 60 min, MPP⁺ levels in animals administered saline + MPTP were twice those measured in animals given DSP-4 + MPTP. Two hours after MPTP administration, small amounts of MPP⁺ were detected in both groups of animals. No cerebellar MPP⁺ was found at 4, 6 or 12 h after MPTP administration in either group of animals (table 4).

**Discussion**

In this study we found that DSP-4 (50 mg/kg i.p., 3 days after administration) produces a pattern of NE loss that follows the pattern of innervation of LC neurons without changing striatal DA levels when injected alone. The loss of NE exacerbated MPTP-induced striatal DA depletion 7 days after MPTP administration. By contrast, despite the fact that the same pattern of NE depletion was observed, we did not find any such enhancement when DSP-4 was administered 12 h after MPTP. The enhancement of MPTP toxicity produced by a previous lesion of LC axon terminals did not rely on significant modifications in the striatal kinetics of MPTP/MPP⁺. In particular, no significant differences were detected in the levels of MPTP or MPP⁺ within the striatum of animals treated with MPTP compared with DSP-4 + MPTP-injected mice. It is likely that NE depletion induced by DSP-4 did not modify striatal MPTP entry, because the striatal amounts of MPTP at 10 min after systemic MPTP administration were similar in NE-depleted and control animals. There were no differences in the early formation of MPP⁺, because at 30 min after systemic administration, there was a complete disappearance of MPTP in both groups of animals, and an equal amount of striatal MPP⁺ was measured. Only slight differences were observed between the two groups of animals in the time to reach the striatal concentration peak. These data could be attributed to the high ratio between striatal DA and NE terminals, which makes it unlikely that a few NE axons within the striatum could segregate a significant amount of MPP⁺. However, general effects (basal metabolism, cardiovascular activity and body temperature) potentially altered by the loss of central NE axons did not modify the striatal kinetics of MPTP/MPP⁺.

It is well established that the primary event in MPTP toxicity involves the formation of the toxic metabolite MPP⁺ by monoamine oxidase B (MAO-B) (Chiba et al., 1984; Markey et al., 1984). Nigrostriatal DA axons take up MPP⁺ through the high-affinity plasma membrane DA transporter (Javitch and Snyder, 1984). Despite controversies concerning downstream mechanisms (Tipton and Singer, 1993), the initial phase, consisting of MPP⁺ formation, is a milestone in the mechanism of MPTP toxicity. This is confirmed by the fact that compounds known to protect from MPTP toxicity also reduce the concentration of MPP⁺ within the striatum (Heikkila et al., 1984; Markey et al., 1984; Irwin et al., 1987). This concept led to the assumption that striatal MPP⁺ levels represented a predictable index of MPTP toxicity. Similarly, species differences in MPTP toxicity were interpreted as the consequence of differential striatal MPP⁺ half-life (Johannessen et al., 1985; Riachi et al., 1988). Though originally accepted as correct, the concept has been slightly modified during the last few years as a consequence of further evidence that differences in MPTP/MPP⁺ kinetics contribute to, but are not sufficient to account for, DA neurotoxicity (Giavelli et al., 1994a; Zuddas et al., 1994; Fornai et al., 1995a; Vaglini et al., 1996). In a recent study (Vaglini et al., 1996), we provided evidence indicating that ex vivo MPP⁺ level does not necessarily correlate with MPTP-induced striatal DA depletion after different combined treatments, including DA uptake inhibitors. Indeed, it is likely that despite similar or paradoxically increased bioavailability of striatal MPP⁺, the combined administration of these drugs changes the physiology of the dopaminergic terminals, inducing a pharmacological antagonism to the effects of MPP⁺.

In line with these recent reports, our findings indicate that the enhancement of MPTP toxicity achieved by NE depletion in LC does not result from differences in the striatal kinetics. In the present study, we observed more marked differences in the nigral MPTP/MPP⁺ kinetics; however, this difference in the pattern of the time course did not result in significant modifications of the total amount of nigral MPP⁺ measured by the area under the curve (AUC).

Paradoxically, the nigral MPP⁺ peak was higher in saline + MPTP-treated animals than in DSP-4 + MPTP-injected mice. This indicates that chronic NE loss enhances the neurotoxic effect induced by MPTP/MPP⁺ via mechanisms other than increase in the nigrostriatal amount of the neurotoxin. On this basis, it could be postulated that the exacerbation of MPTP-induced parkinsonism in LC-lesioned animals might result from an impaired recovery of nigrostriatal DA terminals. However, in this study, we observed that the more marked DA depletion in DSP-4-pretreated animals described 7 days after MPTP (Marien et al., 1993) does not occur if the NE loss is produced when MPP⁺ has already
This suggests that NE depletion needs to occur before MPTP administration in order to enhance MPTP-induced striatal DA loss. Moreover, we observed an increased MPTP-induced acute (6 h and 12 h) striatal DA depletion in DSP-4-treated animals compared with saline-injected controls. Although the more marked acute DA depletion does not necessarily reflect increased neurotoxicity, this is an indication that more pronounced effects are induced acutely by MPTP in NE-depleted than in control mice.

Previous studies (Pileblad et al., 1984; Chan et al., 1993) have shown that MPTP causes acute DA depletion in the mouse striatum, and this has been suggested to occur as the consequence of a pharmacological rather than a toxic effect of MPP⁺. Nonetheless, a more pronounced acute DA depletion in DSP-4-pretreated mice indicates that in the absence of LC fibers, nigrostriatal DA neurons possess a different pharmacological sensitivity to equal amounts of striatal MPTP/MPP⁺ compared with control animals. Taken together, these data indicate that the loss of LC NE innervation enhances the chronic striatal DA depletion observed 7 days after MPTP and increases the acute effects of the DA neurotoxin but does not impair the recovery of striatal DA during this time interval. This acute enhancement is not related to significant modifications in the striatal levels of MPTP or MPP⁺, as evidenced by data obtained at seven different time intervals. It could be argued that the method used in this study (i.e., post-mortem striatal MPP⁺ assay) did not account for the amount of MPP⁺ that is really available for the dopaminergic synaptic terminal. It is well known that MPP⁺ can be stored in a variety of neurons and in glial cells as well. An increased post-mortem amount of striatal MPP⁺ does not provide information about the specific cellular and subcellular compartment within the striatum that is retaining the toxic metabolite. Indeed, it is likely that despite a similar bioavailability of striatal MPP⁺, NE depletion changes the physiology of DA terminals (Andén and Grabowska, 1976), causing a pharmacological enhancement of the effects of MPP⁺. For instance, Giovanni et al. (1994 a, b) found that similar amounts of MPP⁺ were able to increase the striatal DA release 40-fold in mice, whereas this increase was much more limited (3-fold) in rats. This acute pharmacological difference was related to a more pronounced DA toxicity in mice than in rats. Our data are consistent with these findings, because we observed a more marked acute DA depletion persisting at 12 h in DSP-4 + MPTP-treated animals than in MPTP-injected controls. These findings, combined with our failure to observe any MPTP enhancement when NE depletion was induced 12 h after MPTP administration, suggest that in NE-depleted animals, a more pronounced acute neuronal sensitivity to MPTP occurs. The mechanism by which this acute enhancement might occur is presently unknown, but one possible explanation involves the fact that NE agonists increase mRNA levels for fibroblast growth factor, which prevents MPTP toxicity (Otto and Unsicker, 1990; Follesa and Mocchetti, 1993). The loss of the NE system in the SN might be crucial in this connection. However, although NE-containing varicosities arising from the LC are known to project through the SN pars compacta (Mason and Fibiger, 1979), there is no electron microscopic evidence that these fibers actually synapse in this area. Similarly, functional studies on the role of nigral NE have never been carried out.

In any case, the loss of trophic effects after a noradrenergic lesion should be involved more in the long-term survival of dopaminergic neurons than in the acute response to MPTP that has been described in the present work.

Previous studies showed a marked retention of MPP⁺ within the NE terminals (Herkenham et al., 1991). In the present study, we found considerable modifications in the kinetics of MPTP/MPP⁺ in those areas where massive NE depletion occurred after the administration of DSP-4. These modifications never involved the levels of MPTP/MPP⁺ 10 min after MPTP administration, which suggests that the NE lesion influences neither the delivery of MPTP nor the early, faint formation of MPP⁺. By contrast, we observed a longer persistence of MPTP in those areas where massive NE depletion occurred; this effect was accompanied by (and probably consequent to) a delay in MPP⁺ formation and a reduction in MPP⁺ peak. In the hippocampus in particular, where the most prominent NE depletion was observed, there was an earlier MPP⁺ formation and clearance in saline + MPTP-treated mice compared with DSP-4-injected animals. The same trend was observed in other brain regions in which substantial NE depletion occurred. These data indicate that the loss of the NE system delays the time course of MPTP/MPP⁺ kinetics. This effect is totally opposite to the hypothesis that an intact NE system would have significantly contributed to the local uptake and segregation of MPP⁺, and it was unexpected in view of previous data showing a dense retention of MPP⁺ within the NE system, which has been described in areas outside the domain of the nigrostriatal dopaminergic pathway (Herkenham et al., 1991). Indeed, such modifications were not detected in those areas (striatum and hypothalamus) where DSP-4 did not produce any significant effect.

In conclusion, our data confirm that LC NE loss worsens MPTP-induced experimental parkinsonism. This effect is not related to mechanisms that occur after MPTP/MPP⁺ clearance, because the NE loss produced as soon as MPP⁺ was eliminated did not modify the DA-depleting effect induced by MPTP. By contrast, we observed a marked increase in MPTP-induced acute DA depletion in DSP-4-pretreated animals. In this study, we show that despite the pivotal role of NE terminals in taking up and storing MPP⁺, MPTP enhancement does not depend on modifications in the striatal kinetics of MPTP/MPP⁺.

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