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Norepinephrine loss exacerbates methamphetamine-induced striatal dopamine depletion in mice

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Abstract

Evidence is accumulating that norepinephrine depletion enhances the neurotoxic effect of the parkinsonism inducing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In this study we investigated whether norepinephrine loss potentiates methamphetamine-induced striatal dopamine depletion. Injection of C57BL/6N mice with methamphetamine (2 × 5 mg/kg i.p., at 2-h intervals) produced only a partial (50%) striatal dopamine depletion 7 days after drug administration. Pretreatment with the selective noradrenergic neurotoxin *N*-(-2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4; 50 mg/kg i.p.) enhanced methamphetamine-induced striatal dopamine depletion by 86%, without decreasing striatal dopamine levels when injected alone. Our results extend previous findings obtained with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in DSP-4-pretreated mice.

Keywords: Locus ceruleus; Methamphetamine; Striatal dopamine; DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine)

1. Introduction

The impairment of the central noradrenergic system in neurodegenerative disorders and particularly in Parkinson's disease has been well documented by several neuropathological studies (for a review, see Alvord and Forno, 1992). Biochemical studies performed post mortem confirmed the co-existence of a massive norepinephrine depletion with dopamine loss in parkinsonian patients (Hornykiewicz and Kish, 1986). Although the significance of this alteration remains unknown, recent evidence suggests a causative role for norepinephrine depletion in increasing the susceptibility of the nigrostriatal dopaminergic pathway to neurotoxic insults.

In particular, norepinephrine depletion caused by damage to locus coeruleus neurons enhances striatal dopamine depletion induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in both monkeys (Mavridis et al., 1991) and mice (Marien et al., 1993) and nigral dopaminergic cell loss in monkeys (Mavridis et al., 1991) and mice (Bing et al., 1994) as well. These data were obtained either by lesioning the noradrenergic perikarya with a focal injection (within the locus coeruleus) of 6-hydroxydopamine (Mavridis et al., 1991; Bing et al., 1992) or by damaging noradrenergic axons via systemic administration of *N*-(-2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) (Marien et al., 1993). To extend these observations, in this study we pre-treated C57BL mice with the noradrenergic neurotoxin DSP-4, which induces a selective pattern of norepinephrine loss involving noradrenergic terminals arising from the locus coeruleus (Ross, 1976; Jonsson et al., 1981; Delini-Stula et al., 1984; Hallman and Jonsson, 1984; Grzanna et al., 1989). We then administered methamphetamine at a dose which by itself moderately reduced striatal dopamine levels, to evaluate whether DSP-4 pretreatment was able to enhance the methamphetamine-induced striatal dopamine depletion.

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2. Materials and methods

Male C57/6N Black mice (Charles River, Calco, CO, Italy), 8–9 weeks old, weighing 20–24 g, were kept under environmentally controlled conditions (12-h light/dark cycle with light on between 07.00 and 19.00 h; room temperature 21°C) with food and water ad libitum. Animals were treated according to the National Institutes of Health Guiding Principles in the Care and Use of Laboratory Animals.

Mice were treated intraperitoneally (i.p.) with a single dose of DSP-4 hydrochloride (Sigma Chemical Co., St. Louis, MO, USA, 50 mg/kg). Seven days after DSP-4 administration, methamphetamine (Sigma Chemical Co., St. Louis, MO, USA, 5 mg/kg) was administered i.p. twice, with a 2-h interval. Given the high variability of methamphetamine toxicity (Sonsalla, personal communication), the dose of methamphetamine (5 mg/kg × 2) was selected from the results of pilot studies performed with different doses and schedules of administration, in order to produce consistently an intermediate degree of striatal dopamine depletion.

Control groups received either saline, methamphetamine, or DSP-4 alone, at the same doses and times used for the group given the combined treatment. Seven days after DSP-4 injection, a group of animals treated with DSP-4 together with a group of saline-injected controls were killed to assay cortical and striatal monoamine levels at the time of methamphetamine administration. Seven days after methamphetamine treatment (14 days after DSP-4), the remaining animals were killed. Animals were killed by cervical dislocation, the brains were immediately removed, and the frontal cortex and the striatum were dissected and processed for the measurement of dopamine, serotonin, norepinephrine and metabolites. The frontal cortex was carefully dissected at the level of the frontal pole.

Briefly, the cortex and the striatum were sonicated in 0.6 ml of ice-cold 0.1 M perchloric acid containing 10 ng/ml of 3,4-dihydroxybenzylamine (Sigma) as the 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 a high-pressure liquid chromatography (HPLC) system where dopamine, serotonin and metabolites were analyzed as previously described (Fornai et al., 1993). Briefly, the HPLC system consisted of a precolumn, a C18 reverse-phase column (Beckman, San Ramon, CA, USA) and an ESA coulometric electrochemical detector. The mobile phase contained, in 1 liter of deionized distilled water, 1250 mg of heptanesulphonic acid, 120 mg of EDTA, 6 ml of phosphoric acid (85%), 10 ml of triethylamine and 20 ml of acetonitrile. The potentials applied were +0.35 and −0.35 V for oxidizing and reducing electrodes, respectively. Each compound was recorded at both electrodes and the amount of each neurotransmitter was measured based upon two different regression curves (the first for peaks integrated by the oxidizing electrode and the second for peaks integrated by the reducing electrode). A standard curve was prepared using known amounts of norepinephrine, dopamine, serotonin and their metabolites (Sigma), dissolved in 0.1 M perchloric acid containing a constant amount (10 pg/μl) of the internal standard (3,4-dihydroxybenzylamine), as used for tissue samples. The standard curve for each compound (monoamine or its metabolites) was determined by regression analysis of the ratios of the peak areas (compound area/3,4-dihydroxybenzylamine area) for various concentrations of each compound recorded at the reducing electrode. For each study, results were obtained from six animals per group, each assayed in duplicate. Each experiment has been replicated at least twice, and therefore the data are expressed as the means ± S.E.M. from 12 animals per group. The effects of DSP-4 and methamphetamine treatment on striatal and cortical monoamine levels were statistically evaluated using analysis of variance with Scheffe's post-hoc analysis. The null hypothesis was rejected when $P < 0.05$.

3. Results

At the time of methamphetamine administration, 7 days after DSP-4 pre-treatment, the levels of serotonin and dopamine in the cortex and the striatum were not different between DSP-4- and saline-treated mice, whereas the levels of norepinephrine in the frontal cortex were markedly reduced (Table 1). Seven days after methamphetamine administration, animals injected with methamphetamine alone revealed a significant dopamine reduction compared with saline-in-

Table 1
Monoamine levels in the striatum and the frontal cortex 7 days after DSP-4

	Striatum (ng/mg protein)		Frontal cortex (ng/mg protein)	
	Saline	DSP-4	Saline	DSP-4
DA	127.8 ± 9.3	120.0 ± 8.9	0.81 ± 0.06	0.77 ± 0.07
NE			5.1 ± 0.3	1.1 ± 0.04 [a]
5-HT	4.7 ± 0.5	4.0 ± 0.7	5.8 ± 0.2	6.2 ± 0.5

Animals were killed 7 days after DSP-4 (50 mg/kg) administration. The brain was quickly removed and the striatum was dissected and processed for monoamine analysis. Dopamine (DA), norepinephrine (NE) and serotonin (5-HT) were assayed by high-pressure liquid chromatography. Values are expressed as the means + S.E.M. for 12 animals per group, each assayed in duplicate. [a] $P < 0.05$ compared with mice treated with saline.

jected controls (60.0 ± 2.3 ng/mg protein and 120.5 ± 5.6 ng/mg protein, respectively). Animals pre-treated with DSP-4 and then given methamphetamine displayed a significant enhancement of methamphetamine-induced dopamine depletion (18.8 ± 1.5 ng/mg protein) (Fig. 1A).

Similarly to animals killed 7 days after DSP-4, no change was detected in striatal and frontocortical serotonin levels in animals treated with DSP-4 alone 14 days after treatment (Table 2), whereas cortical norepinephrine levels were reduced by 19.6% and 22.5% in mice given DSP-4 alone and DSP-4 + methamphetamine, respectively (Fig. 1B). No significant reduction was detectable in frontocortical dopamine levels 7 days after treatment with methamphetamine alone or with DSP-4 + methamphetamine (0.70 ± 0.06 ng/mg and 0.72 ± 0.08 ng/mg protein, respectively, compared to 0.77 ± 0.06 ng/mg protein of controls). Only a slight, non-significant reduction in frontocortical and striatal serotonin levels was detected 7 days after treatment with methamphetamine alone or with DSP-4 + methamphetamine (Table 2).

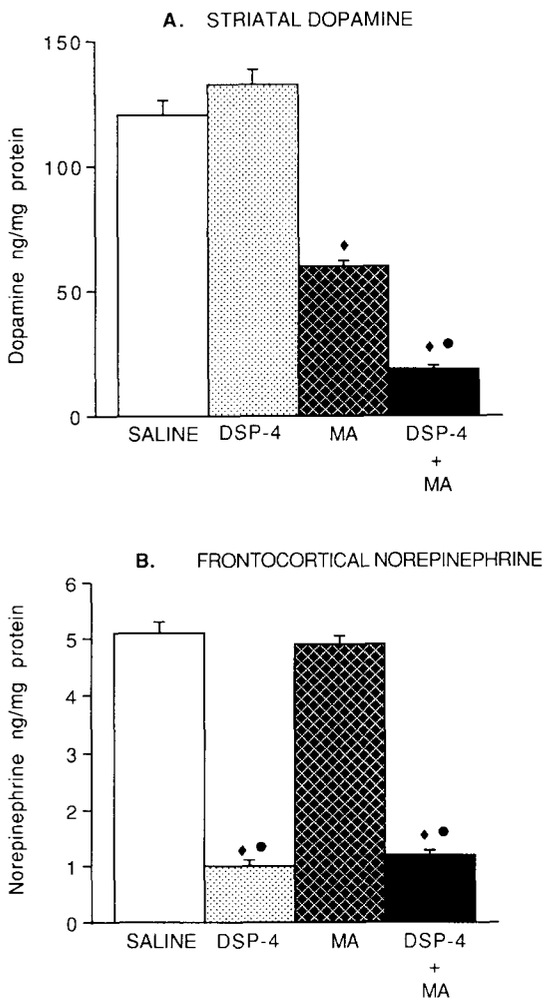

Fig. 1. (A) Dopamine levels in the striatum and (B) norepinephrine levels in the frontal cortex of C57BP/6N mice. Animals were killed 7 days after methamphetamine (MA, 5 mg/kg×2; 2 h apart) and 14 days after DSP-4 (50 mg/kg) administration. Values are expressed as the means ± S.E.M. for 12 animals per group. ♦ $P < 0.05$ compared with saline-injected controls, • $P < 0.05$ compared with mice treated with methamphetamine alone.

Table 2
Serotonin levels 7 days after methamphetamine administration (14 days after DSP-4)

	Striatum (ng/mg protein)	Frontal cortex (ng/mg protein)
Saline	5.1 ± 0.6	6.2 ± 0.4
DSP-4	4.2 ± 0.5	5.8 ± 0.6
MA	4.3 ± 0.5	6.0 ± 0.5
DSP-4 + MA	4.0 ± 0.4	5.8 ± 0.8

Serotonin levels in the striatum and the frontal cortex of C57Bl/6N mice. Animals were killed 7 days after methamphetamine (MA, 5 mg/kg×2; 2 h apart) and 14 days after DSP-4 (50 mg/kg) administration. The brain was quickly removed and the striatum was dissected and processed for monoamine analysis. Values are expressed as the means ± S.E.M. for 12 animals per group.

4. Discussion

Methamphetamine toxicity is extremely variable depending on the species, strain and age of animals (Seiden and Ricaurte, 1987). In these series of experiments, we consistently found that the double administration (2 h apart) of methamphetamine (5 mg/kg i.p.) to 8-week-old C57/BL mice produced an intermediate degree of striatal dopamine depletion compared with control values. This study shows that pretreatment of C57/BL mice with the noradrenergic neurotoxin DSP-4 (50 mg/kg) markedly enhances the striatal dopamine depletion induced by methamphetamine.

Although contrasting data have been reported concerning the effects of DSP-4 on cortical and striatal serotonin levels (Ross, 1976; Dooley et al., 1983), in our experimental conditions DSP-4 consistently did not modify the striatal or cortical serotonin content 7 and 14 days after treatment.

It is therefore likely that the permanent norepinephrine depletion induced by DSP-4 is responsible for the enhancement of striatal dopamine loss induced by methamphetamine.

These data extend recent findings obtained in the same strain of mice by Marien et al. (1993). In this study, the authors found that pretreatment with DSP-4 enhanced the striatal dopamine depletion induced by the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. The reason for this effect is still unknown. Previous studies failed to measure the striatal levels of the toxic metabolite 1-methyl-4-phenylpyridinium after lesion of locus coeruleus noradren-

ergic axons. It is therefore possible that the enhancement of MPTP toxicity induced by DSP-4 could in fact be related to the reduction of active uptake sites for 1-methyl-4-phenylpyridinium within the noradrenergic terminals. This relative decrease of noradrenergic vs. dopaminergic uptake could increase the amount of the neurotoxin available for lesioning the nigrostriatal dopaminergic axons. In a similar way, in our experimental conditions, damage to noradrenergic axons by DSP-4 could have significantly increased the uptake of methamphetamine and amphetamine by the nigrostriatal dopaminergic terminals. Although further studies exploring this possibility are necessary, according to Marien et al. (1993) we have to admit that compared with the high density of dopaminergic axons in the rodent striatum, noradrenergic terminals are rarely present, if at all (Jones and Moore, 1977) and are thus unlikely to compete significantly for the uptake of dopaminergic neurotoxins. In addition, the dopamine depletion induced by neurotoxins is not prevented or attenuated by drugs acting as norepinephrine-uptake inhibitors (Javitch and Snyder, 1985; Melamed et al., 1985).

Apart from the mechanisms underlying the enhancement by DSP-4 of methamphetamine-induced striatal dopamine loss, these data confirm that norepinephrine loss may enhance neurotoxic damage to nigrostriatal dopaminergic neurons.

In previous studies, methamphetamine has been reported to cause a substantial serotonin depletion both in the striatum and in the frontal cortex of rodents (Ricaurte et al., 1980). However, in our experiments we consistently found that a dose of 5 mg/kg of methamphetamine did not significantly reduce serotonin levels in these brain areas. This could be due either to the low dosage of methamphetamine we used, and/or to the lower sensitivity of C57 Black mice to the serotonin-depleting effects of methamphetamine.

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