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Biochemical Effects of the Monoamine Neurotoxins DSP-4 and MDMA in Specific Brain Regions of MAO-B-Deficient Mice

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ABSTRACT Previous studies reported that drugs acting as monoamine oxidase (MAO)-B inhibitors prevented biochemical effects induced by the neurotoxins N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) and 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). In this study, we administered DSP-4 (50 mg/kg) or MDMA (50 mg/kg ×2, 2 h apart) to MAO-B deficient mice. Monoamine content in various brain regions (cerebellum, frontal cortex, hippocampus, hypothalamus, striatum, substantia nigra) was assayed 1 week after neurotoxin administration. Injection of DSP-4 to wild-type mice caused a marked norepinephrine (NE) loss in specific brain regions. Unexpectedly, DSP-4 caused similar effects in MAO-B-deficient and in wild-type mice in all brain regions investigated. These results suggest that MAO-B is not involved in DSP-4 toxicity. In wild-types, the neurotoxin MDMA induced both serotonin (5HT) and dopamine (DA) depletion in specific brain areas. In MAO-B-deficient mice, 5HT depletion observed in wild-types did not occur. In contrast, MDMA produced a more pronounced DA loss in knockout mice compared with wild-types. The present findings, together with previous data obtained using selective enzyme inhibitors, suggest that MAO-B is not involved in the mechanism of action of DSP-4, whereas it plays opposite roles in MDMA-induced DA and 5HT depletions. **Synapse 39:213–221, 2001.**

INTRODUCTION

The physiological significance of monoamine oxidase (MAO)-B in the central nervous system (CNS) remains largely unknown. While studies carried out in mice and rats established that MAO-A plays a central role in metabolizing endogenous monoamines such as serotonin (5HT), norepinephrine (NE), and dopamine (DA) (Kato et al., 1986; Cases et al., 1995), MAO-B in rodents does not affect levels of these amines (Kato et al., 1986; Finberg et al., 1995; Grimsby et al., 1997). Nonetheless, MAO-B activity seems to be crucial for the metabolism of the trace amine phenylethylamine (PEA) (Lamensdorf et al., 1996; Grimsby et al., 1997) and neurotoxins like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Heikkila et al., 1984), N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4; Gibson, 1987), or methylenedioxymethamphetamine (MDMA; Sprague and Nichols, 1995a,b). The role of MAO-B in MPTP toxicity is well established since MAO-B inhibitors protect against MPTP-induced striatal DA depletion (Heikkila et al., 1984). Consistently, MAO-B deficient mice are resistant to MPTP toxicity (Grimsby et al., 1997). In contrast, evidence concerning the involvement of MAO-B in DSP-4- or MDMA-induced monoamine depletion is based solely on experiments carried out by using enzyme inhibitors. These experiments indicate that a few MAO-B blockers prevent the biochemical alterations induced by DSP-4 (Gibson, 1987) or MDMA (Sprague and Nichols, 1995a,b). In order to verify the potential role of MAO-B activity in the mech-

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anism of action of these neurotoxins, we administered these neurotoxins to MAO-B-deficient mice.

The neurotoxin DSP-4 is known to reduce NE within the CNS (Landa et al., 1984). While in mice this effect is limited to NE, in rats a significant 5HT depletion has been observed (Fornai et al., 1996). The damage induced by DSP-4 has been related to the formation of a cyclic compound (azyridinium ion), which in turn would be metabolized by MAO-B and taken up by NE terminals. These conclusions were drawn since the selective and irreversible MAO-B inhibitor l-deprenyl prevents chronic NE loss induced by DSP-4 (Gibson, 1987). However, another selective MAO-B inhibitor (MDL 72974) failed to protect against DSP-4 toxicity (Finnegan et al., 1990).

Since l-deprenyl and its metabolites (i.e., amphetamines) also block DSP-4 uptake, it was postulated that protective effects of l-deprenyl may not rely on the inhibition of MAO-B (Finnegan et al., 1990; Finnegan, 1993).

Recent studies used new MAO-B inhibitors to prevent the biochemical effects induced by DSP-4 (Yu et al., 1994a,b, 1995; Zhang et al., 1996). These new compounds neither produce amphetamine-like metabolites nor affect the uptake mechanisms, despite inducing significant neuroprotection against DSP-4. These studies reevaluated the role of MAO-B in DSP-4 toxicity. To test this hypothesis, in the present work we administered DSP-4 to MAO-B-deficient mice.

In the second part of the study, we investigated the role of MAO-B in mediating the biochemical effects induced by MDMA. The neurotoxin MDMA ("ecstasy") produces 5HT depletion in selected brain areas in various animal species (Schmidt, 1987; Finnegan et al., 1988; Ricaurte et al., 1988; O'Hearn et al., 1988). Recent studies by Ricaurte and collaborators demonstrated persistent alterations in central 5HT neurons in humans chronically abusing MDMA (McCann et al., 1998). Therefore, understanding the mechanism of action of MDMA might lead to develop new drugs able to rescue or to prevent this neurotoxicity.

Previous studies have shown that MDMA-induced chronic 5HT loss requires the activity of MAO-B (Schmidt, 1987; O'Hearn et al., 1988). This is based on the protective effects of MAO-B inhibitors. To verify the validity of this hypothesis, we treated MAO-B-deficient mice with MDMA. In mice, MDMA, apart from 5HT loss, induces DA depletion (Cadet et al., 1995; Ali et al., 1991; Logan et al., 1988). Therefore, administering MDMA to MAO-B-deficient mice will enable us to understand the role of this enzyme in both DA and 5HT depletions induced by MDMA.

MATERIALS AND METHODS

Housing and selection of mice for the experiment

MAO-B-deficient mice used in the present experiment were generated as previously described (Grimsby et al., 1997). Previous studies have shown that MAO-B activity in wild-type mice increases with age (Grimsby et al., 1997); therefore, all mice used in the study were 15–17 weeks old. Mice were kept under controled conditions (12-h light/dark cycle with lights on between 0700 and 1900). Since the toxicity of amphetamine derivatives is highly variable, depending critically on room temperature (Albers and Sonsalla, 1995) and the number of animals per cage (for review, see Seiden and Ricaurte, 1987; Wagner et al., 1981; Vargas-Rivera et al., 1990), we carefully maintained a constant room temperature (21°C) and humidity (60%) both in housing and in performing the experiments. The number of animals per cage ($n = 10$) and the size of the cage (38×22 cm wide and 15 cm high) were the same as previously published (Fornai et al., 1999a). Animals were handled in accordance with the Guidelines for Animal Care and Use developed by the National Institutes of Health.

Experimental design and drugs administration

We determined the levels of NE, DA, 5HT, and their metabolites in various brain regions of MAO-B-deficient and wild-type mice 7 days after administration of the neurotoxins DSP-4 or MDMA. We administered DSP-4 (RBI, Natick, MA) at a dose (50 mg/Kg), which has been shown to produce the maximal NE depletion in C57 Black mice (Fornai et al., 1996). MDMA (Sigma Chemical Co., St. Louis, MO) was administered at a dose of 50 mg/Kg two times, 2 h apart, which has been shown to produce depletion of both DA and 5HT in mice.

Drug solutions were freshly prepared in 0.9% NaCl.

Using this experimental protocol, we administered either DSP-4 or MDMA to both MAO-B knockout and wild-type mice. Mice were sacrificed 7 days after treatment by cervical dislocation. The brain was quickly removed and placed in ice-cold saline. The following regions were dissected according to the original method of Glowinski and Iversen (1966): cerebellum, frontal cortex, hippocampus, hypothalamus, striatum, and substantia nigra.

HPLC analysis

NE, DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5HT, and 5-hydroxyindolacetic acid (5HIAA) were measured by HPLC with a reversed phase column (250×4.5 mm, ODS C18, SGE) and two coulometric electrochemical detectors as previously described (Fornai et al., 1997). The mobile phase consisted of a citrate-phosphate buffer (0.04 M citric acid, 0.06 M $Na_2HPO_4 \cdot 2H_2O$) containing 0.1 mM EDTA, 0.6 mM 1-heptanesulphonic acid sodium salt, and 10% methanol.

Data analysis

For catecholamine assays, a standard curve was prepared using known amounts of DA and metabolites (Sigma) dissolved in perchloric acid (0.1 M) containing a constant amount (10 pg/μL) of the internal standard (DBA; Sigma). The standard curve for each compound (DA, NE, 5HT, or metabolites) 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. Results from wild-type and MAO-B knockout mice are expressed as the mean ± SE of values obtained from groups of 10 mice. The effects of each treatment on DA, NE, 5HT, and their metabolites were compared using ANOVA with Sheffè post-hoc analysis. Null hypothesis (H_0) was rejected when $P < 0.05$.

RESULTS

Basal monoamine levels in different brain areas

As reported in a previous study (Grimsby et al., 1997), the levels of monoamines in the cerebellum, frontal cortex, hippocampus, hypothalamus, striatum, and substantia nigra did not differ between wild-types and knockout mice (Figs. 1–6). The levels of metabolites between saline-injected wild-type and MAO-B-deficient mice were similar (data not shown), confirming that in basal condition MAO-B does not play a detectable role in monoamine metabolism (Grimsby et al., 1997; Fornai et al., 1999b).

Effects of monoamine neurotoxins in different brain areas

Cerebellum

Among the three monoamines assayed in this study only NE was detectable in the cerebellum. Administration of the neurotoxin DSP-4 produced significant NE loss both in wild-type (0.38 ± 0.02 ng/mg protein) and in MAO-B knockout mice (0.43 ± 0.03 ng/mg protein) compared to saline-injected groups (3.07 ± 0.24 ng/mg protein and 3.25 ± 0.29 ng/mg protein, respectively). In contrast, MDMA did not modify the levels of this neurotransmitter in either group of mice (Fig. 1).

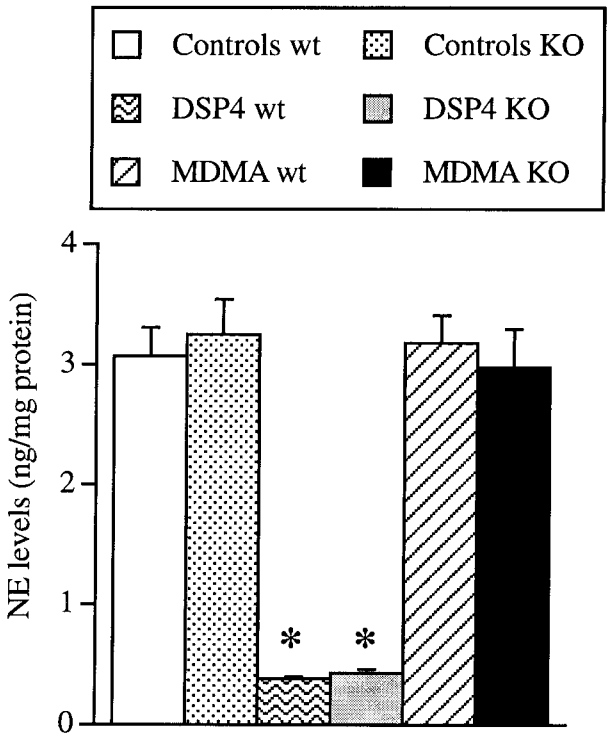


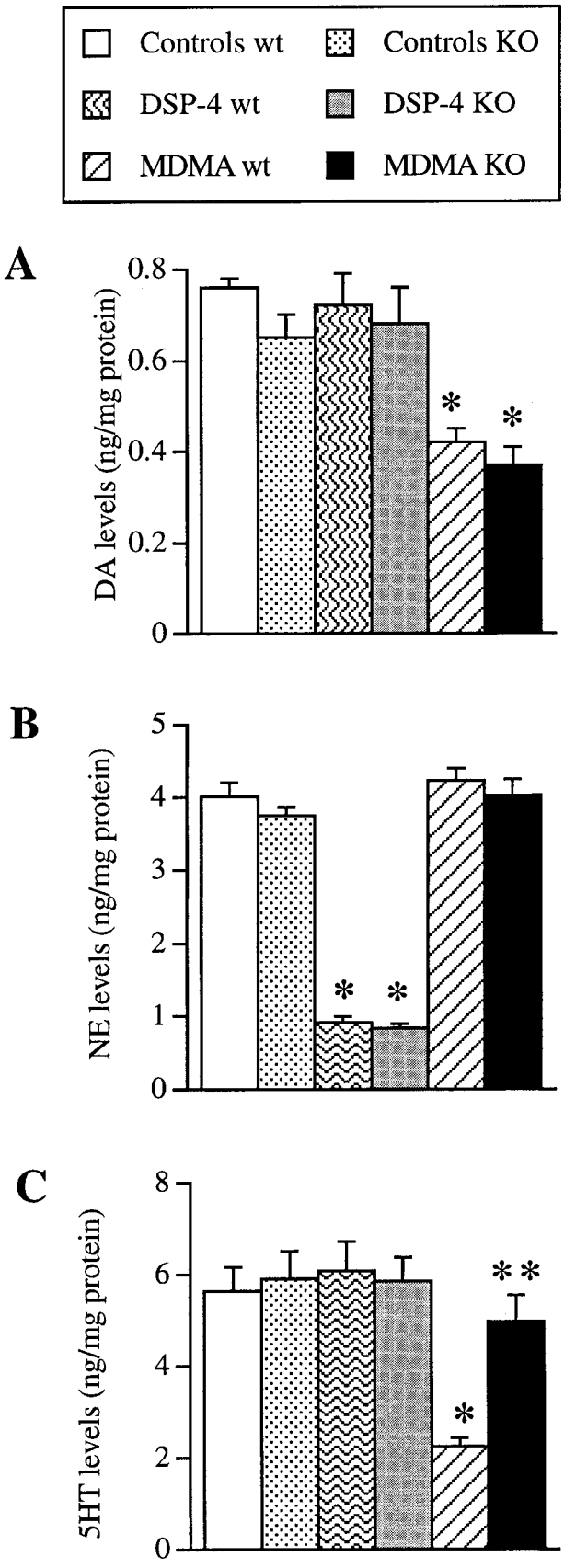
Fig. 1. Monoamine levels in the cerebellum. Norepinephrine (NE) levels were assayed in the cerebellum of MAO-B knockout and wild-type mice 7 days after either DSP-4 (50 mg/kg, i.p.) or MDMA (50 mg/kg ×2, 2 h apart, i.p.) administration. DSP-4 produced a significant NE loss both in MAO-B knockout and in wild-type compared with saline-injected mice (controls). No effect was produced by MDMA. Dopamine and serotonin levels were under detection limit. Each group was composed of 10 animals. Comparisons between groups were performed using ANOVA with Sheffè post-hoc analysis. *$P < 0.05$ compared with saline-injected controls.

Frontal cortex

The neurotoxin DSP-4 (50 mg/Kg) did not produce any significant change in DA levels in the frontal cortex of either wild-type and MAO-B-deficient mice (Fig. 2A). In contrast, MDMA produced a significant DA depletion in both wild-type animals (0.42 ± 0.03 ng/mg protein) and in MAO-B knockout mice (0.37 ± 0.04 ng/mg protein) compared with their controls (0.76 ± 0.02 ng/mg protein and 0.65 ± 0.05 ng/mg protein, respectively) (Fig. 2A). NE levels were markedly reduced in DSP-4-treated mice (both wild-type, 0.91 ± 0.08 ng/mg protein, and MAO-B knockout, 0.83 ± 0.06) compared with saline-injected animals (wild-type: 4.01 ± 0.19 ng/mg protein and MAO-B knockout: 3.75 ± 0.12 ng/mg protein), whereas MDMA did not modify frontocortical NE level (Fig. 2B). In DSP-4-treated animals 5HT levels were similar to saline-injected mice, whereas administration of MDMA produced a significant 5HT depletion in wild-type which was not observed in knockouts (Fig. 2C).

Hippocampus

DA levels in the hippocampus of DSP-4-treated mice were similar to controls both for wild-type and knockout mice. DA levels were slightly reduced in MDMA-treated wild-type and in MAO-B knockout mice, but the decrease was not significant (Fig. 3A). The levels of NE in the hippocampus were mostly affected by the neurotoxin DSP-4 both in wild-type and in knockout mice. As espected, MDMA did not modify NE levels in this brain area in either group of mice (Fig. 3B). DSP-4 did not modify 5HT levels in any group of mice, whereas MDMA produced a 5HT depletion which occurred only in wild-type, being 5HT levels of MAO-B knockout similar to controls (Fig. 3C).



Hypothalamus

DA levels in the hypothalamus were not affected either by DSP-4 or by MDMA administration, either in MAO-B knockout mice or in wild-type (Fig. 4A). There was a slight, nonsignificant decrease of NE levels in the hypothalamus of DSP-4-treated MAO-B-deficient mice and wild-type. MDMA administration did not produce significant changes in NE levels in either group of mice (Fig. 4B). Similarly, the levels of 5HT in the hippocampus were not modified by DSP-4 or by MDMA administration in either group of mice (Fig. 4C).

Striatum

Whereas the neurotoxion DSP-4 did not produce any effect on striatal DA levels in either group of animals, MDMA administration produced a significant decrease in striatal DA levels of wild-type mice (64.32 ± 4.50) which was exacerbated in MAO-B knockout mice (37.44 ± 3.87 ng/mg protein) compared to controls (94.68 ± 8.55 ng/mg protein and 102.84 ± 10.91 ng/mg protein, respectively) (Fig. 5A). Levels of NE in the striatum were below the detection limit. Striatal 5HT levels were not affected by DSP-4 administration in either group of mice (Fig. 5B). In contrast, MDMA produced a significant decrease in striatal 5HT levels in wild-type mice (1.77 ± 0.11 ng/mg protein) compared with wild-type injected with saline (2.40 ± 0.13 ng/mg protein). The decrease in striatal 5HT was completely prevented in MAO-B knockout mice (2.81 ± 0.21 ng/mg protein, similar to MAO-B knockout mice injected with saline: 2.96 ± 0.351 ng/mg protein). The results suggest MAO-B is involved in MDMA-induced 5HT depletion.

Substantia nigra

As shown in Figure 6A, DSP-4 did not produce a significant effect in DA levels of either group of mice. In contrast, MDMA induced a significant decrease of DA levels in the substantia nigra of wild-type and such a reduction was enhanced in MAO-B-deficient animals (Fig. 6A). Administration of DSP-4 produced a moderate but significant decrease in NE levels in substantia nigra in wild-type (2.25 ± 0.21 ng/mg protein) and MAO-B knockout mice (2.26 ± 0.11 ng/mg protein) compared with the same groups injected with saline (wild-type, 4.01 ± 0.33 ng/mg protein, and knockouts, 4.56 ± 0.36 ng/mg protein) (Fig. 6B). As usual, no

Fig. 2. Monoamine levels in the frontal cortex. Dopamine (DA) (**A**), norepinephrine (NE) (**B**), and serotonin (5HT) (**C**) levels were evaluated in the frontal cortex of MAO-B knockout and wild-type mice 7 days after either DSP-4 (50 mg/kg, i.p.) or MDMA (50 mg/kg ×2, 2 h apart, i.p.) administration. Each group was composed of 10 animals. Comparisons between groups were performed using ANOVA with Sheffè post-hoc analysis. $^{*}P < 0.05$ compared with saline-injected controls. $^{**}P < 0.05$ compared with MDMA-injected wild-type (MDMA wt).

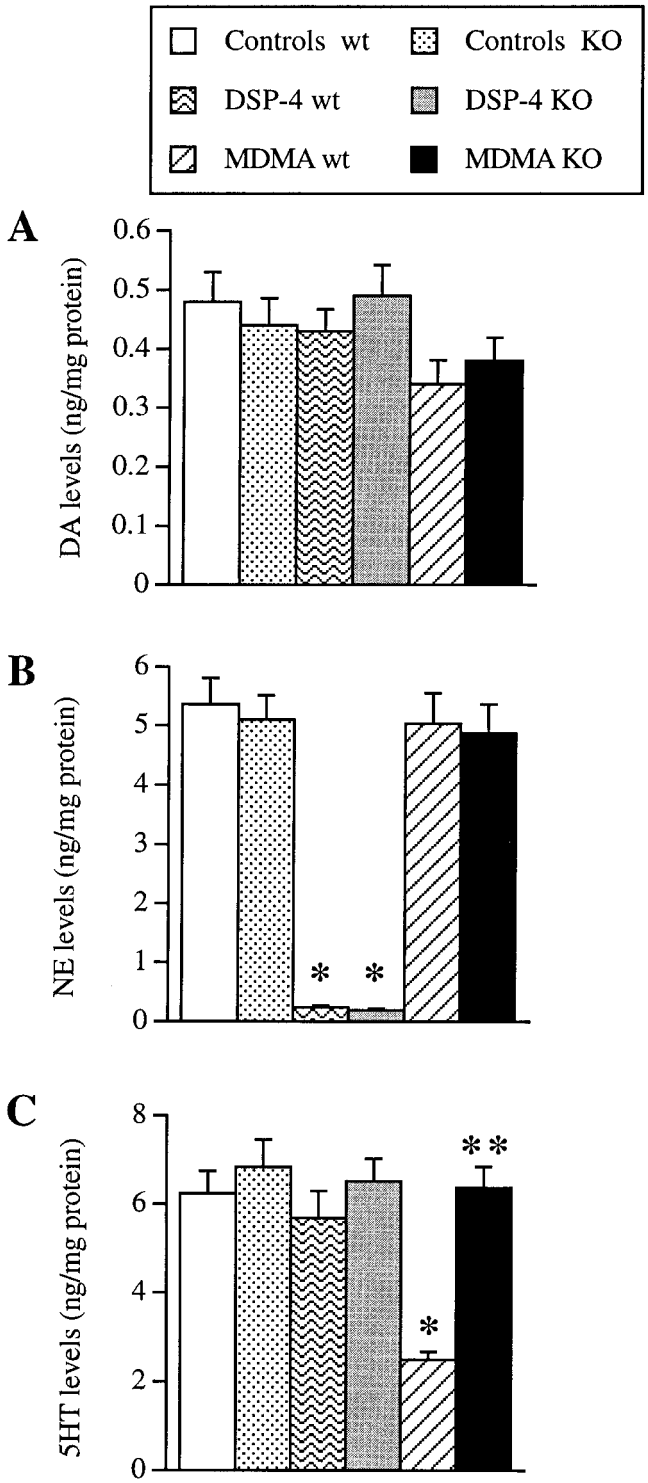


Fig. 3. Monoamine levels in the hippocampus. Dopamine (DA) (**A**), norepinephrine (NE) (**B**), and serotonin (5HT) (**C**) levels were evaluated in the hippocampus of MAO-B knockout and wild-type mice 7 days after either DSP-4 (50 mg/kg, i.p.) or MDMA (50 mg/kg ×2, 2 h apart, i.p.) administration. Each group was composed of 10 animals. Comparisons between groups were performed using ANOVA with Sheffè post-hoc analysis. *$P < 0.05$ compared with saline-injected controls. **$P < 0.05$ compared with MDMA-injected wild-type (MDMA wt).

effects were produced by MDMA on NE levels in any group (Fig. 6B).

As shown in Figure 6C, DSP-4 did not affect 5HT levels in the substantia nigra. In contrast, there was a reduction of nigral 5HT in MDMA-injected wild-type; however, such a 5HT depletion was completely prevented in MAO-B knockouts (Fig. 6C).

DISCUSSION

In the present study we measured monoamine levels in different brain regions of MAO-B-deficient and wild-type mice after exposure to the neurotoxins DSP-4 and MDMA. Confirming a previous report (Grimsby et al., 1997), we found that in basal conditions the levels of DA, NE, and 5HT measured in various brain areas were similar in MAO-B knockout and in wild-type mice.

In this study, we found that only 5HT depletion induced by MDMA in specific brain areas of wild-type (frontal cortex, striatum, substantia nigra, and hippocampus) was abolished in MAO-B-deficient mice. In contrast, the DA depletion produced by MDMA was either similar or more pronounced in MAO-B knockouts compared with wild-type. Finally, DSP-4-induced NE depletion observed in MAO-B knockout mice was similar to wild-types in all brain regions investigated.

Previous studies using the MAO-B inhibitor l-deprenyl suggested that MAO-B is involved in the neurotoxic effects of DSP-4. This was based on the finding that pretreatment with l-deprenyl prevents the biochemical effects of DSP-4 on NE terminals (Gibson, 1987). However, further studies suggested that deprenyl-induced neuroprotection against DSP-4-induced NE loss was unrelated to MAO-B inhibiton (Finnegan et al., 1990; Finnegan, 1993). In particular, using the selective MAO-B inhibitor MDL 72974, no protective effects were observed against DSP-4 toxicity. Similarly, the time course of the protective effects induced by deprenyl appeared to be unrelated to the time course of MAO-B inhibiton. Together, these results led us to hypothesize that the protective effects of l-deprenyl against DSP-4 toxicity were due either to the blockade of DSP-4 uptake by amphetamine (a metabolite of l-deprenyl) or to other mechanisms. Nonetheless, recent studies using two MAO-B inhibitors belonging to the class of aliphatic N-propargylamines demonstrated that pretreatment with these compounds fully prevented the DSP-4-induced NE depletion (Yu et al., 1994a,b, 1995; Zhang et al., 1996). These compounds are highly potent and selective MAO-B inhibitors devoid of any amphetamine-like properties. Therefore, they do not interfere with DSP-4 uptake within NE terminals. These MAO-B inhibitors exerted a significant protection against chronic NE loss induced by DSP-4. Therefore, if MAO-B inhibition protects against DSP-4, we expect that neurotoxicity in the NE system would have been suppressed or at least significantly

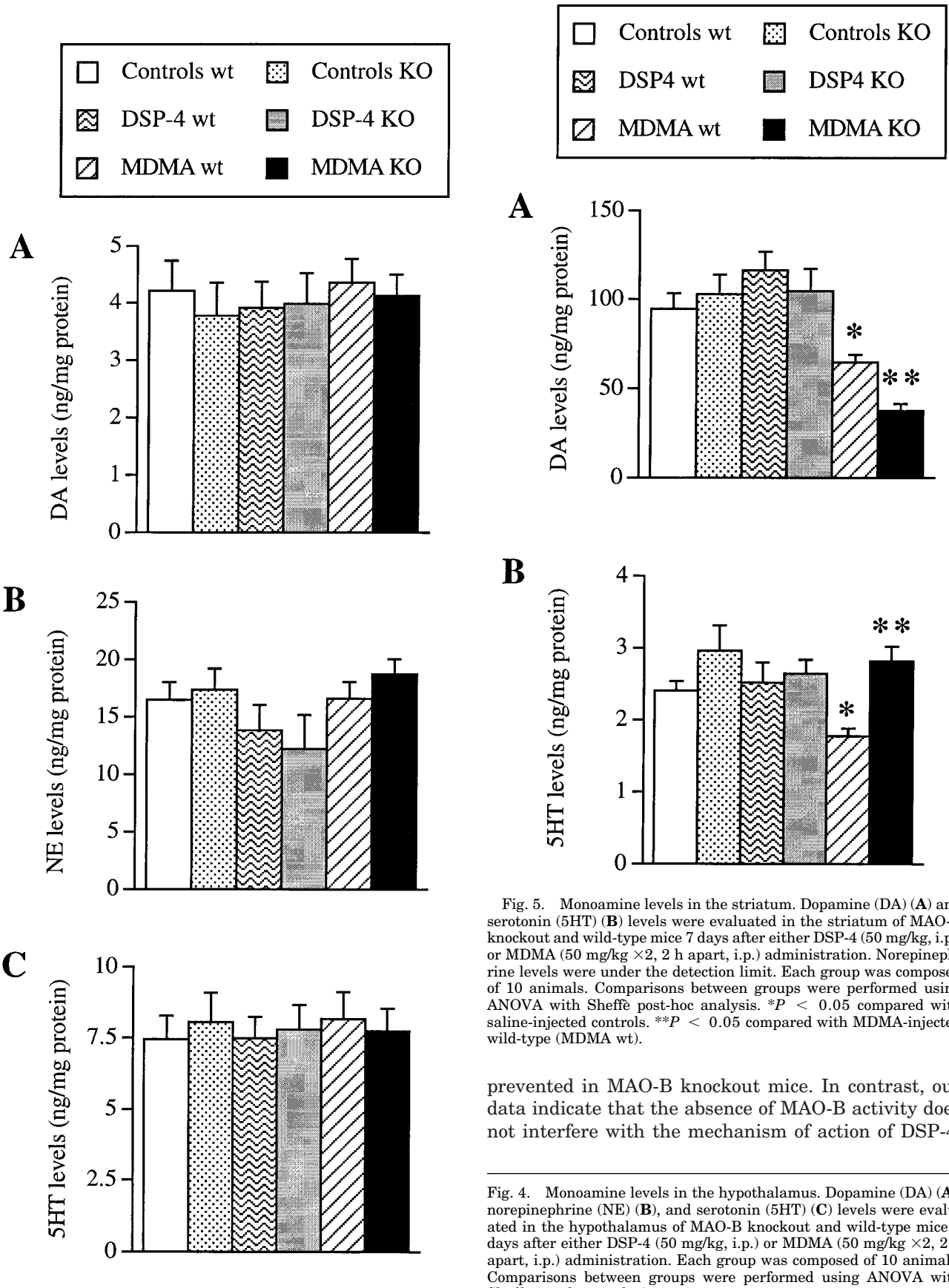
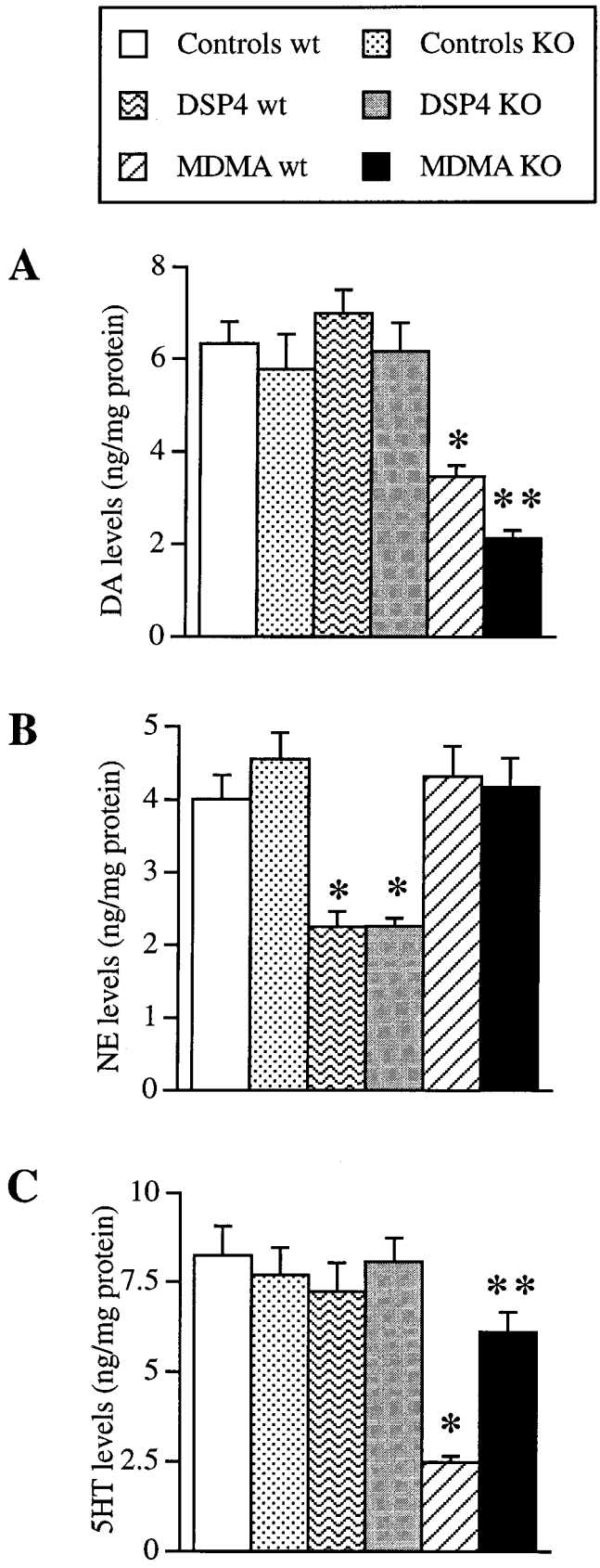


Fig. 5. Monoamine levels in the striatum. Dopamine (DA) (**A**) and serotonin (5HT) (**B**) levels were evaluated in the striatum of MAO-B knockout and wild-type mice 7 days after either DSP-4 (50 mg/kg, i.p.) or MDMA (50 mg/kg ×2, 2 h apart, i.p.) administration. Norepinephrine levels were under the detection limit. Each group was composed of 10 animals. Comparisons between groups were performed using ANOVA with Sheffè post-hoc analysis. $^{*}P < 0.05$ compared with saline-injected controls. $^{**}P < 0.05$ compared with MDMA-injected wild-type (MDMA wt).

prevented in MAO-B knockout mice. In contrast, our data indicate that the absence of MAO-B activity does not interfere with the mechanism of action of DSP-4.

Fig. 4. Monoamine levels in the hypothalamus. Dopamine (DA) (**A**), norepinephrine (NE) (**B**), and serotonin (5HT) (**C**) levels were evaluated in the hypothalamus of MAO-B knockout and wild-type mice 7 days after either DSP-4 (50 mg/kg, i.p.) or MDMA (50 mg/kg ×2, 2 h apart, i.p.) administration. Each group was composed of 10 animals. Comparisons between groups were performed using ANOVA with Sheffè post-hoc analysis.



This could be due to compensatory mechanisms taking place in this enzymatic defect. However, it is unlikely that such a compensatory adaptation might occurr via MAO-A increased activity since this enzyme was found to be in the same range as in wild-type mice (Grimsby et al., 1997). Alternatively, protective effects obtained by administering several (but MDL) MAO-B inhibitors should be ascribed to other properties unrelated to the blockade of the enzyme (Finnegan, 1993; Finberg et al., 1998). This latter hypothesis would explain why not all MAO-B inhibitors are able to prevent DSP-4 induced NE depletion, and it would fit with the time course of deprenyl-induced protection against DSP-4 toxicity (Finnegan, 1993). Neuroprotective effects obtained with MAO-B inhibitors have been claimed for neuronal protection in brain ischemia, brain trauma, or other brain injuries less related to monoaminergic neurons (Speiser et al., 1998; Huang et al., 1999). This neuroprotection remains unclear concerning the mechanism of action and it is beyond the aim of this article. Nonetheless, our data on DSP-4 toxicty in MAO-B knockout mice indirectly call for more in-depth studies to elucidate other pharmacological effects achieved with MAO inhibitors (for review, see Yu, 1994; Magyar et al., 1998).

Confirming previous results, in the present study we found that MDMA-induced chronic 5HT loss requires the activity of MAO-B. This is based on the protective effects of a few MAO-B inhibitors (Schmidt, 1987; O'Hearn et al., 1988) and is in line with the suppression of MDMA-induced 5HT depletion we obtained here in various brain areas of MAO-B knockout mice. The mechanism of action of MDMA has been recently investigated (Sprague and Nichols, 1995a,b; Sprague et al., 1998). In particular, it has been shown that MDMA toxicity is related to the marked release of DA promoted by this neurotoxin. It was speculated that DA is then taken up by 5HT neurons where it is metabolized by MAO-B, thereby generating toxic compounds (Sprague et al., 1998). This would explain why either genetically or pharmacologically inhibiting the activity of MAO-B leads to prevention of MDMA-induced 5HT toxicity.

This hypothesis could be further confirmed by analyzing intracellular 5HT levels within 5HT neurons; in fact, MAO-B inhibitors produce an increase of intraneuronal 5HT and enhancement of MDMA-induced 5HT release (Gu and Azmitia, 1993). Indeed, previous studies have demonstrated a correlation between seroto-

Fig. 6. Monoamine levels in the substantia nigra. Dopamine (DA) (**A**), norepinephrine (NE) (**B**), and serotonin (5HT) (**C**) levels were evaluated in the substantia nigra of MAO-B knockout and wild-type mice 7 days after either DSP-4 (50 mg/kg, i.p.) or MDMA (50 mg/kg $\times 2$, 2 h apart, i.p.) administration. Each group was composed of 10 animals. Comparisons between groups were performed using ANOVA with Sheffè post-hoc analysis. $^{*}P < 0.05$ compared with saline-injected controls. $^{**}P < 0.05$ compared with MDMA-injected wild-type (MDMA wt).

nergic toxicity and the amount of intracellular 5HT levels (Berger et al., 1989). Intracellular analysis of MAO-B within 5HT neurons could also rule out whether in MAO-B knockout mice a regional compensatory effect is taking place. Nonetheless, as stated above, in previous studies (Grimsby et al., 1997; Fornai et al., 1999b) we found that in MAO-B knockout mice there is no compensatory increase in MAO-A activity.

As mentioned in the Introduction, despite the common belief that considers MDMA a selective 5HT neurotoxin, evidence has accumulated indicating that in rodents MDMA possesses a DA-depleting effect which is at least as great as the 5HT-induced depletion (Gough et al., 1991). In particular, in mice the DA loss persists for weeks after treatment and, indeed, represents a marker of MDMA-induced DA neurotoxicity (Cadet et al., 1995; Ali et al., 1991; Logan et al., 1988). In the present study we confirmed that MDMA produces a chronic striatal DA depletion in mice. Moreover, we found that, in contrast to the effects on the 5HT system, the DA-depleting effect was enhanced in the striatum and the substantia nigra of MAO-B knockout mice. These results confirm the hypothesis that 5HT and DA toxicity induced by amphetamine derivatives follow different biochemical pathways (Haughey et al., 1999). This is also in line with previous observations by Riacaurte et al. (1983), who found that fluoxetine, despite preventing 5HT toxicity induced by methamphetamine, increased striatal DA loss induced by the drug.

Indeed, it is not surprising that MDMA-induced DA loss in MAO-B knockout mice was more pronounced than in wild-type. For instance, previous studies reported that MAO nonselective and MAO-B selective inhibitors exacerbate the neurotoxicity of methamphetamine for the DA system (Wagner and Walsh, 1991; Kita et al., 1995). Considering the various similarities among the mechanism of action of various amphetamine derivatives (Metzger et al., 1998) our data suggest that, similar to other amphetamines, MDMA-induced DA toxicity is exacerbated by the suppression of MAO-B activity. This could be the consequence of increased formation of toxic quinones or cysteinyl derivative within the DA terminals in the presence of high DA concentration when MAO activity is reduced. Indeed, MDMA is a potent inhibitor of the isoenzyme MAO-A, which is the isoform contained in the DA terminals (Leonardi and Azmitia, 1994). This latter hypothesis, although suggested by several findings, remains speculative and needs to be tested in further experimental studies.

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