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The Monoamine Oxidase-B Inhibitor L-Deprenyl Protects Against 3,4-Methylenedioxymethamphetamine-Induced Lipid Peroxidation and Long-term Serotonergic Deficits[1]

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ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA)-induced serotonergic neurotoxicity was assessed in the striatum, hippocampus and frontal cortex of rats by using [^{3}H]paroxetine binding to label serotonin (5-HT) uptake sites and 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels as markers of serotonergic function. MDMA (40 mg/kg) induced a significant decrease in both [^{3}H]paroxetine binding B_{max} and 5-HT and 5-HIAA levels 7 days after treatment. The monoamine oxidase-B inhibitor L-deprenyl (2 mg/kg) administered 30 min before MDMA blocked these decreases. MDMA (40 mg/kg) also maximally increased the formation of thiobarbituric acid reactive substances (an indicator of lipid peroxidation) 12 hr after treatment in all three brain regions studied. This increase in malondialdehyde formation was also blocked by pretreatment with L-deprenyl. Tryptophan hydroxylase (TPH) activity was also significantly reduced 18 hr after MDMA. L-Deprenyl reversed this decrease in TPH activity. Another experiment confirmed that a significant fraction of [^{3}H]dopamine uptake into hippocampal synaptosomes was blocked by 500 nM fluoxetine, a selective 5-HT uptake inhibitor. These data suggest that the deamination by monoamine oxidase-B of excessive dopamine within the 5-HT terminal generates hydrogen peroxide that may lead to membrane lipid peroxidation, and perhaps other oxidative insults, resulting in selective 5-HT terminal degeneration subsequent to MDMA treatment.

The substituted amphetamine MDMA ("Ecstasy") is known to selectively deplete 5-HT and 5-HIAA levels in the brain of rats and nonhuman primates after a single high dose or repetitive treatments (McKenna and Peroutka, 1990). The number of 5-HT uptake sites also is decreased after MDMA treatment (Habert *et al.*, 1985; Johnson and Nichols, 1989; Marcusson *et al.*, 1988). Investigations into the mechanism by which MDMA induces this selective serotonergic terminal degeneration have provided evidence of a role for both 5-HT (Berger *et al.*, 1992) and DA (Nash, 1990).

Berger *et al.* (1992) suggested that 5-HT or a metabolite of 5-HT (*e.g.*, 5,6- or 5,7-dihydroxytryptamine) may be the responsible toxin. However, recent studies have shown that MDMA-induced (Sprague *et al.*, 1994) and PCA-induced (Bradberry *et al.*, 1993) neurotoxicity could be prevented by pretreatment of rats with tryptophan or 5-hydroxytryptophan, precursors for the synthesis of 5-HT. This finding suggests that 5-HT or a metabolite of 5-HT is not the ultimate toxin.

Support for a role of DA in MDMA-induced neurotoxicity comes from research in a number of laboratories. Stone *et al.* (1988) have shown that treatment of rats with alpha-methyl-*para*-tyrosine, a DA synthesis inhibitor, or with GBR-12909, a DA uptake blocker, could also protect against MDMA-induced neurotoxicity. Schmidt *et al.* (1990) showed that if DA terminals are destroyed with 6-hydroxydopamine, serotonergic terminal degeneration does not occur. Increased DA synthesis (Nash *et al.*, 1990) and release (Nash, 1990) also occur after MDMA treatment. Nash and Nichols (1991) further showed that serotonergic neurotoxicity was linearly correlated with the acute increase in extracellular DA. Pretreatment with L-DOPA, the DA synthesis precursor, has also been shown to potentiate MDMA-induced neurotoxicity (Schmidt *et al.*, 1991).

Although the role of 5-HT in this neurotoxic process is unclear, the depletion of 5-HT from the serotonergic terminal has been speculated to increase the vulnerability of the ter-

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HPLC, high-performance liquid chromatography; MAO-B, monoamine oxidase-B; MAO-A, monoamine oxidase-A; PCA, *para*-chloroamphetamine; s.c., subcutaneous; i.p., intraperitoneal; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; TPH, tryptophan hydroxylase.

minal to the toxin (Sprague *et al.*, 1994). However, 5-HT release and depletion alone are not sufficient to lead to serotonergic terminal degeneration (Johnson and Nichols, 1991). 5-Methoxy-6-methyl-2-aminoindan is a potent and selective releaser and uptake inhibitor of 5-HT *in vitro* (Johnson *et al.*, 1991) that acutely depletes brain 5-HT *in vivo* (Johnson and Nichols, 1991); given alone, it does not induce serotonergic toxicity. However, when given in combination with (+)-amphetamine, a DA releaser, serotonergic neurotoxicity occurs (Johnson and Nichols, 1991). Furthermore, a carrier-mediated uptake event has also been implicated in the neurotoxic process because MDMA-induced neurotoxicity can be attenuated by treatment with fluoxetine, a 5-HT uptake blocker, up to 6 hr after MDMA administration (Schmidt, 1987).

Immunocytochemical studies have shown that central serotonergic terminals primarily contain MAO-B (Levitt *et al.*, 1982; Westlund *et al.*, 1985). Yet, it is known that MAO-B has a higher affinity for DA in preference to 5-HT in human brain (Riederer *et al.*, 1978) and that 5-HT is preferentially deaminated by MAO-A and not MAO-B (White and Tansik, 1979). It has been postulated that the presence of MAO-B in the serotonergic terminal serves a protective role by degrading foreign monoamines or potential false transmitters (Levitt *et al.*, 1982; Pintar *et al.*, 1983). However, there are no definitive studies that address this issue.

We have hypothesized that after MDMA treatment, DA may be transported into the monoamine-depleted 5-HT terminal, where it is deaminated by MAO-B, generating hydrogen peroxide. If this process were excessive, it seemed possible that the reductive capacity of the neuron might be overwhelmed by the increased production of hydrogen peroxide. The resulting membrane lipid peroxidation and oxidative insult might then lead to selective 5-HT terminal degeneration. The present study was designed to test this hypothesis.

Methods

The present study was carried out in accordance with protocols approved by the Purdue University Animal Care and Use Committee.

Materials. MDMA hydrochloride was synthesized in our laboratory following the methods of Nichols *et al.* (1986). [^{3}H]Paroxetine and [^{3}H]DA were purchased from New England Nuclear (Boston, MA) at a specific activity of 20.5 Ci/mmol. Fluoxetine hydrochloride was generously provided by Eli Lilly & Co. (Indianapolis, IN). L-Deprenyl was purchased from Research Biochemicals International (Natick, MA). TBA and all other reagents used were purchased from Sigma Chemical Co. (St. Louis, MO).

Animal treatment and sample collection procedure. Adult, male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 175 to 199 g were used in all studies. Rats were individually housed under standard environmental conditions (14 hr/10 hr light/dark cycle; 24°C) and had *ad libitum* access to food and water.

In the first series of experiments, 42 animals were divided into four treatment groups. Rats were injected with saline, MDMA (40 mg/kg s.c.), L-deprenyl (2 mg/kg i.p.) or L-deprenyl 30 min before MDMA. Half of the animals from the saline, MDMA and L-deprenyl plus MDMA treatment groups were then killed by decapitation 3 hr after treatment. All remaining animals were killed 7 days after treatment. The striatum, hippocampus and frontal cortex were then dissected out and stored at −70°C until assay for [^{3}H]paroxetine binding or 5-HT and 5-HIAA analysis.

In the second series of experiments, 24 animals were randomly allocated to one of four treatment groups. Rats received MDMA (40 mg/kg s.c.) or saline. The animals were then killed by decapitation 8, 12 or 18 hr after treatment. Three saline-treated animals were also killed at each time point, and these groups were compared. There was no difference between the saline-treated groups, and the data from all 9 animals were pooled to serve as the control. The striatum, hippocampus and frontal cortex were processed immediately for determination of MDA formation.

In the third series of experiments, 24 rats were randomly divided into one of four treatment groups. Rats then received saline, MDMA (40 mg/kg s.c.), L-deprenyl (2 mg/kg i.p.) or L-deprenyl 30 minutes before MDMA. Twelve hours after treatment, the animals were killed by decapitation, and the striatum, hippocampus and frontal cortex were removed. Tissue samples were handled as in the second series of experiments until assay for MDA formation.

In the fourth series of experiments, 4 rats were killed by decapitation. The hippocampus was removed, and synaptosomes from this tissue were prepared for [^{3}H]DA uptake studies.

In the fifth series of experiments, 24 rats were randomly allocated into one of four treatment groups: saline, MDMA (40 mg/kg s.c.), L-deprenyl (2 mg/kg i.p.) or the L-deprenyl 30 min before MDMA. The animals were then killed 18 hr after treatment. One hour before decapitation, the animals were treated with NSD-1015, a central aromatic amino acid decarboxylase inhibitor. The accumulation of 5-hydroxytryptophan was then measured using HPLC-electrochemical detection as an assessment of TPH activity.

5-HT uptake carrier measurements. A modified procedure of Marcusson *et al.* (1988) was used to measure [^{3}H]paroxetine binding sites. Because it has been previously reported that only B_{max} and not the K_d value is altered after MDMA treatment (Battaglia *et al.*, 1987), it is possible to estimate the number of 5-HT uptake sites with a single saturating (1 nM) concentration of [^{3}H]paroxetine. Nonspecific binding was determined with 1 μM fluoxetine.

5-HT, 5-HIAA and 5-HTP determinations. Brain regions were sonicated for 15 sec in 50 μl of a solution containing 0.1 M $HClO_4$, 0.05% Na_2EDTA and 0.1% $Na_2S_2O_5$. The samples were then centrifuged at 14,000 × *g* for 4 min with a tabletop centrifuge. The supernatant was collected, and 50 μl was injected onto the HPLC column (Brownlee C18, Anspec, Ann Arbor, MI). A Model 400 EG & G Princeton electrochemical detector (Princeton, NJ) with series dual electrodes was used. The mobile phase consisted of 50 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM Na_2EDTA, 0.034% sodium octyl sulfate and 25% methanol. Peaks were integrated with the Dynamax Methods Manager Software (Rainin, Woburn, MA).

Malondialdehyde assay. MDA, an index of lipid peroxidation, was measured in tissue samples using a modified method of Ohkawa *et al.* (1979). Although MDA appears to be the major substance that reacts with TBA, we have reported the results as TBARS. We added 100 μl of 10% sodium dodecyl sulfate to the sonicated tissue samples for solubilization, followed by 1.3 ml of 0.5% TBA in 20% glacial acetic acid (pH 3.5). The samples were then incubated at 85°C for 60 min. The tubes were cooled and centrifuged at 4300 × *g* for 10 min. The absorbance of the supernatant was measured at 532 nm, and the concentration of TBARS formed was estimated using a molar absorptivity of $1.5 \times 10^5 \cdot M^{-1} \cdot cm^{-1}$.

[^{3}H]DA uptake into hippocampal synaptosomes. A modified procedure of Steele *et al.* (1987) was used. Hippocampal tissue was homogenized in 15 vol of ice-cold 0.32 M sucrose. The homogenate was centrifuged at 1086 × *g* and 4°C for 10 min. The supernatant was then recentrifuged at 17,800 × *g* for 10 min, and the resulting pellet was resuspended in the same volume of sucrose solution. Incubations were carried out in a shaking incubator under an atmosphere of 95% O_2-5% CO_2 at 37°C or at 0°C to measure total tissue uptake and nonspecific uptake, respectively. Five-minute preincubation was begun by adding 0.2 ml of the synaptosomal preparation to test tubes containing 1.70 ml of O_2-saturated Krebs-Henseleit buffer with 100 μM pargyline and 50 μl of H_2O or 50 μl of 2×10^{-5} M fluoxetine (final concentration, 500 nM). [^{3}H]DA (final concentration, 100 nM) was added to the sample mixture; incubations were contin-

ued for 5 min and then terminated by cooling in ice and rapid filtration.

Statistical analysis. Data were analyzed by analysis of variance with a Student-Newman-Keuls post hoc test. When comparison between only two groups was made, a Student's t test was used. Significance was set at $P \leq .05$. All measurements were based on tissue wet weight.

Results

Protection against MDMA-induced neurotoxicity with L-deprenyl. The protective effects of L-deprenyl on MDMA-induced serotonergic terminal degeneration are shown in figure 1. A single dose of MDMA (40 mg/kg) produced a significant reduction in the number of [^{3}H]paroxetine binding sites in the striatum and hippocampus and a nonsignificant decrease ($P = .06$) in the frontal cortex 7 days after treatment. L-Deprenyl reversed these effects.

5-HT and 5-HIAA levels were also significantly reduced 7 days after a single dose of MDMA. L-Deprenyl blocked this decrease in 5-HT and 5-HIAA levels in all brain regions studied.

Effects of MDMA and L-deprenyl on acute 5-HT and 5-HIAA levels. Three hours after treatment with MDMA, 5-HT and 5-HIAA levels were significantly reduced in the hippocampus and frontal cortex, and 5-HIAA levels were significantly reduced in the striatum. L-Deprenyl did not block this acute reduction in 5-HT and 5-HIAA (fig. 2), indicating that the long-term protection by L-deprenyl is not related to acute changes in monoamine levels.

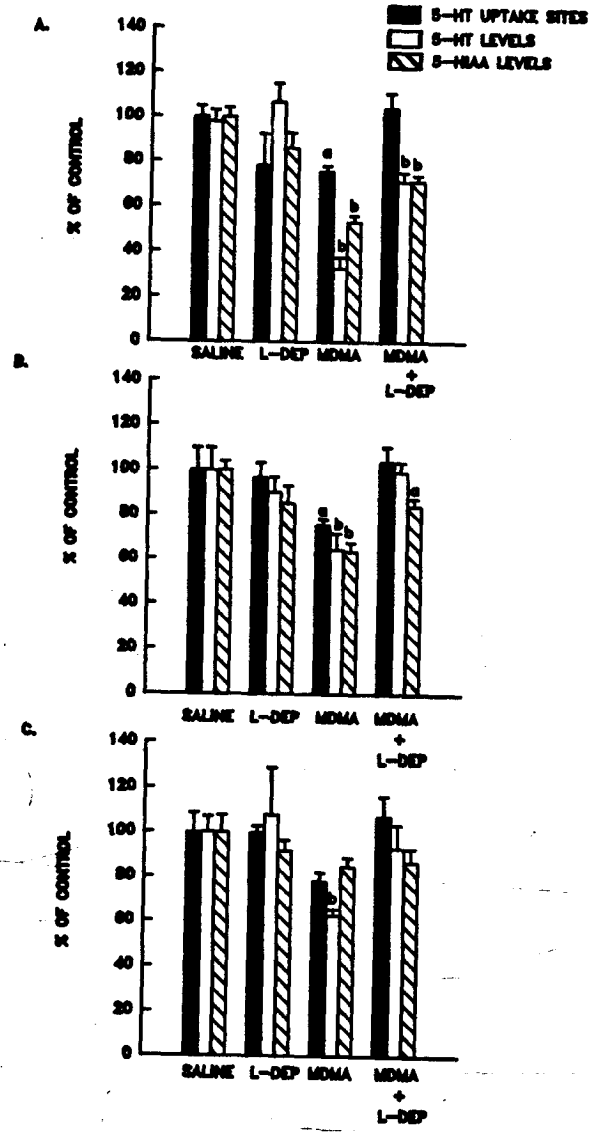

Fig. 1. Effects of MDMA (40 mg/kg) and L-deprenyl (2 mg/kg) on serotonergic parameters 7 days after treatment. 5-HT, 5-HIAA and the uptake (B_{max} value) for striatum (A), hippocampus (B) and frontal cortex (C) are reported as percent of saline control values. Saline control values were striatum: 5-HT, 2157 ± 109 pg/mg; 5-HIAA, 1308 ± 53 pg/mg, uptake, 31.5 ± 1.5 fmol/mg; hippocampus: 5-HT, 654 ± 64 pg/mg; 5-HIAA, 928 ± 30 pg/mg; uptake, 24.9 ± 2.5 fmol/mg; and cortex: 5-HT, 1356 ± 95 pg/mg; 5-HIAA, 703 ± 54 pg/mg; uptake, 23.7 ± 2.1 fmol/mg. Each value is the mean ± S.E.M. for 6 animals. [a] Significantly different from saline control; [b] significantly different from all other groups.

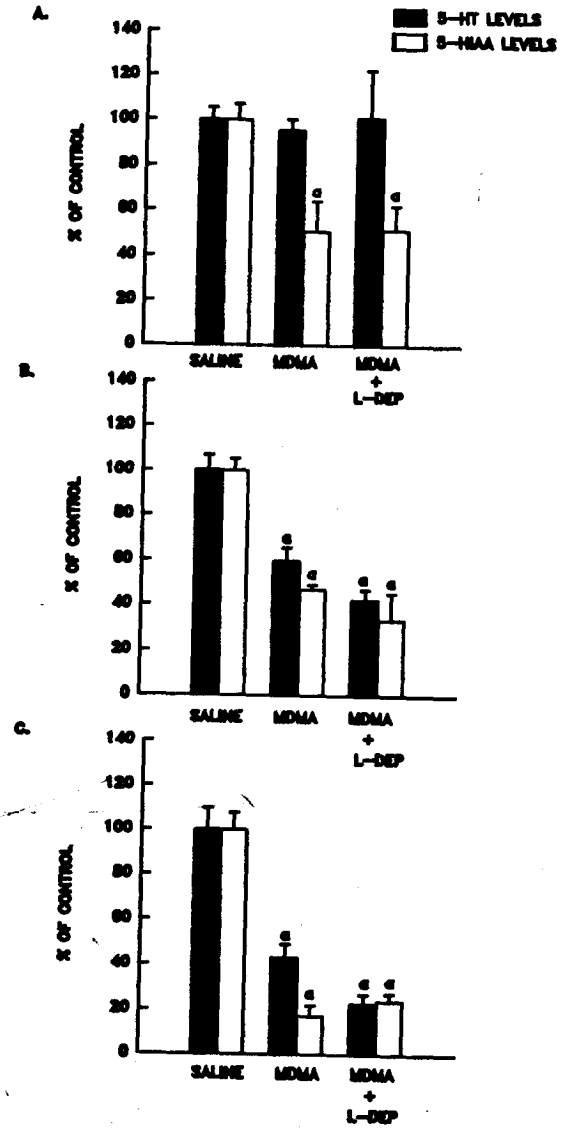

Fig. 2. Effects of MDMA (40 mg/kg) and L-deprenyl (2 mg/kg) on acute 5-HT and 5-HIAA levels 3 hr after treatment. 5-HT and 5-HIAA for striatum (A), hippocampus (B) and frontal cortex (C) are reported as percent of saline control values. Saline control values were striatum: 5-HT, 1470 ± 75 pg/mg; 5-HIAA, 1091 ± 75 pg/mg; hippocampus: 5-HT, 440 ± 30 pg/mg; 5-HIAA, 1005 ± 50 pg/mg; and cortex: 5-HT, 940 ± 30 pg/mg; 5-HIAA, 840 ± 65 pg/mg. Each value is the mean ± S.E.M. for 6 animals. [a] Significantly different from saline control.

MDMA and lipid peroxidation. Figure 3 shows the effect of MDMA on the formation of TBARS 8, 12 and 18 hr after MDMA (40 mg/kg). All three brain regions showed a significant increase in the formation of TBARS at 12 hr. An approximately 60% increase in TBARS formation was observed in the hippocampus and frontal cortex, and a 44% increase was observed in the striatum.

L-Deprenyl protection of MDMA-induced lipid peroxidation. As was observed in the second series of experiments, a single dose of MDMA (40 mg/kg) produced an increase in the formation of TBARS 12 hr after treatment. This increase was significant in the hippocampus and frontal cortex. Although a 40% increase in TBARS formation was seen in the striatum after MDMA treatment, this response was not significant. L-Deprenyl (2 mg/kg) alone had no significant effect on TBARS formation at this time point. Pretreatment with L-Deprenyl 30 min before MDMA blocked the observed increase in TBARS formation in the frontal cortex and attenuated the increase seen in the hippocampus (fig. 4).

[^{3}H]DA uptake into hippocampal synaptosomes. Figure 5 provides evidence that the 5-HT uptake carrier protein in hippocampal synaptosomes is capable of transporting [^{3}H]DA. [^{3}H]DA uptake into these synaptosomes was inhibited by 47% with 500 nM fluoxetine. This concentration is approximately one-30th of the reported K_i value for fluoxetine inhibition of DA uptake *via* the DA transporter (Wong *et al.*, 1974). These results suggest that the 5-HT transport protein can recognize DA as a substrate.

TPH activity after MDMA and L-deprenyl. Treatment with MDMA resulted in 60% decrease in 5-HTP accumulation in the striatum, indicating a significant reduction in TPH activity. L-Deprenyl alone produced a slight (although nonsignificant) increase in 5-hydroxytryptophan levels. Pretreatment with L-deprenyl 30 min before MDMA prevented the decrease in 5-hydroxytryptophan accumulation seen after MDMA alone (fig. 6).

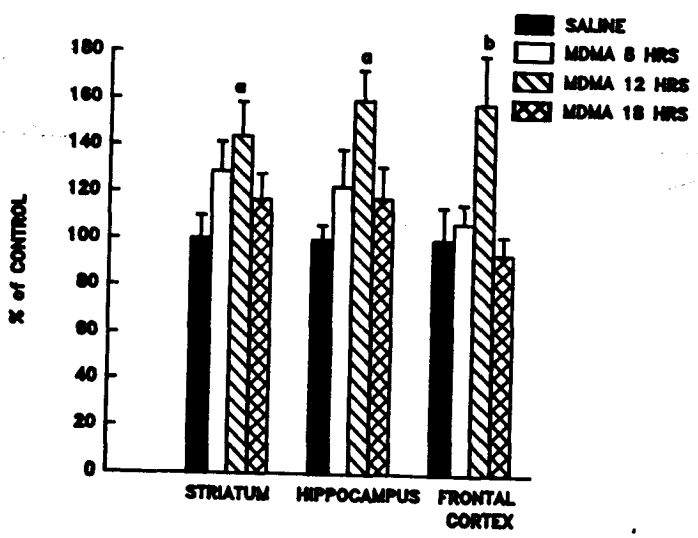

Fig. 3. Time course of MDMA (40 mg/kg) effect on the formation of TBARS. Each value is the mean ± S.E.M. [a] Significantly different from saline; [b] significantly different from all other groups ($P < .05$). Saline control values (ng/g of tissue) were 232.1 ± 22.8 for the striatum, 118.0 ± 7.3 for the hippocampus and 179.7 ± 26.4 for the frontal cortex.

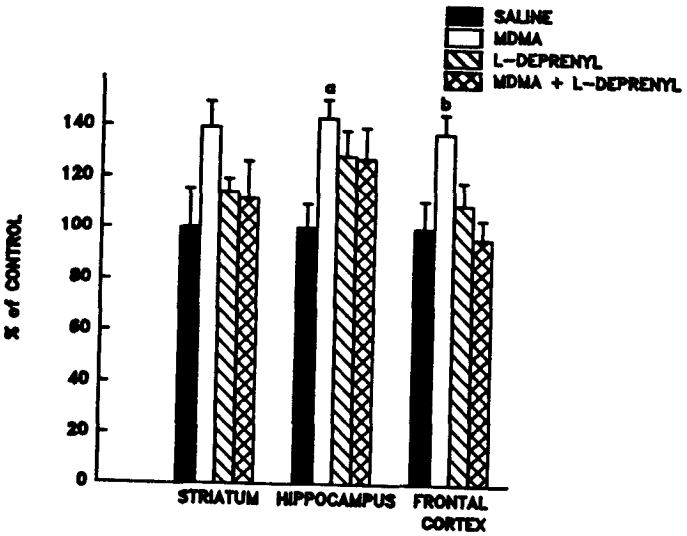

Fig. 4. Effects of MDMA (40 mg/kg) and L-deprenyl (2 mg/kg) on the formation of TBARS 12 hr after treatment. Each value is the mean ± S.E.M. for 6 animals. [a] Significantly different from saline; [b] significantly different from all other groups ($P < .05$). Saline control values (ng/g of tissue) were 262.8 ± 37.3 for the striatum, 178.8 ± 17.1 for the hippocampus and 194.2 ± 24.1 for the frontal cortex.

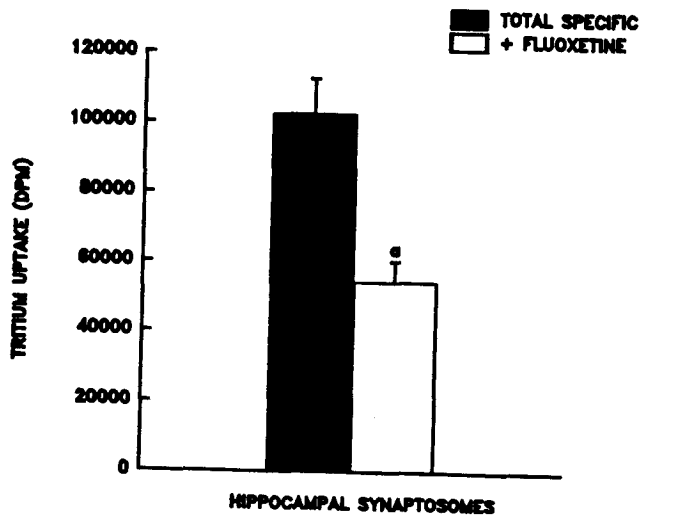

Fig. 5. [^{3}H]DA uptake into hippocampus synaptosomes. Each value is the mean ± S.E.M. for 4 animals. [a] Significantly different from saline control ($P \leq .05$).

Discussion

MDMA has been shown to induce a rapid 5-HT release in rat brain (Nichols *et al.*, 1982; Schmidt *et al.*, 1987; Steele *et al.*, 1987), followed by a subsequent long-term decrease in 5-HT and 5-HIAA levels (Commins *et al.*, 1987; Schmidt *et al.*, 1987). MDMA also leads to activation of the 5-HT$_{2A}$ receptor (Nash *et al.*, 1990; Schmidt *et al.*, 1992), resulting in a marked increase in DA synthesis (Nash *et al.*, 1990). The 5-HT released by MDMA may be responsible for this receptor activation (Huang and Nichols, 1993). The increase in DA synthesis apparently occurs in response to concurrent MDMA-induced DA release and 5-HT$_{2A}$ receptor stimulation (Huang and Nichols, 1993).

For MDMA-induced neurotoxicity to occur, it appears that the 5-HT terminal must first be depleted of its endogenous

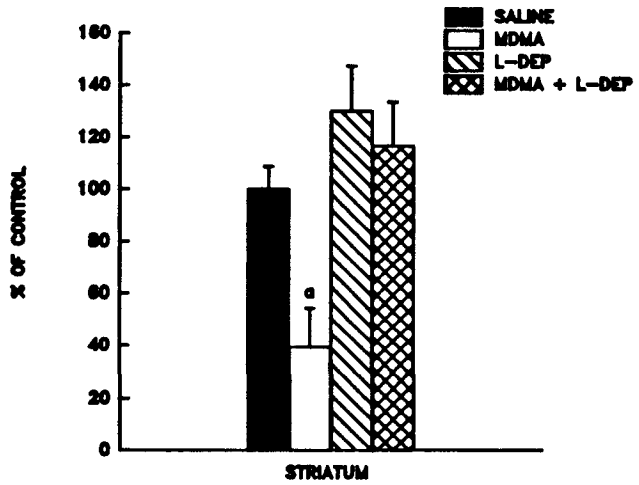

Fig. 6. Effects of L-deprenyl (2 mg/kg) on MDMA-induced reduction of TPH activity in the striatum 18 hr after treatment. 5-HTP values are reported as percent of saline control. Saline control values for striatal 5-HTP were 3698 ± 383 pg/mg. [a] Significantly different from all other groups ($P < .05$). Each value is the mean ± S.E.M. for 6 animals.

5-HT (Sprague *et al.*, 1994). We have speculated that this depletion renders the terminal vulnerable to the uptake of the actual toxin (*e.g.*, DA). Because 5-HT terminals primarily contain MAO-B (Westlund *et al.*, 1985), we hypothesize that deamination of abnormally high levels of DA within the 5-HT terminal may account for terminal degeneration. The present study, showing that inhibition of MAO-B with L-deprenyl (fig. 1) protects against MDMA-induced decreases in [^{3}H]paroxetine binding, is consistent with this hypothesis.

Previously, we reported that L-deprenyl and another MAO-B inhibitor, MDL-72974, attenuated the decreases in [^{3}H]paroxetine binding, 5-HT and 5-HIAA levels seen in the striatum after a single 40-mg/kg dose of MDMA (Sprague and Nichols, in press). Although the inhibition of MAO-B is the primary effect of L-deprenyl and MDL-72974, the possibility of free radical scavenging activity may also account for their protective effects (Knoll, 1988).

The elevated level of extracellular DA is clearly implicated as a possible candidate in the neurotoxicity of MDMA. Evidence for this was first provided when Stone *et al.* (1988) showed that MDMA-induced neurotoxicity could be prevented by inhibition of DA synthesis with alpha-methyl-*para*-tyrosine. Further support came when Schmidt *et al.* (1990) demonstrated that if DA terminals were destroyed with 6-hydroxydopamine, the toxicity of MDMA was attenuated. Schmidt *et al.* (1991) also reported that treatment of rats with the DA precursor L-DOPA potentiated the neurotoxicity. Nash and Nichols (1991) showed that the acute increase in extracellular DA concentration after a single dose of MDMA (20 mg/kg) was negatively correlated with the levels of 5-HT and 5-HIAA 1 week later. Thus, there was compelling evidence for a role of DA in the toxicity of MDMA. Yet, the mechanism by which this DA increase could lead to serotonergic neurotoxicity remained unknown. The results of the present study point to DA as the penultimate toxin, through its deamination by MAO-B resulting in the generation of hydrogen peroxide, the ultimate toxic species.

For these events to occur, DA must be transported into the 5-HT terminal. Waldmeier (1985) reported that in rats treated with amfonelic acid, a DA uptake inhibitor, and haloperidol, a D_2 antagonist known to increase DA turnover, striatal DA was markedly increased, whereas 5-HT levels were significantly reduced. However, if the animals were pretreated with citalopram, a selective 5-HT uptake inhibitor, this decrease in 5-HT was blocked. Waldmeier concluded that the released DA was taken up into 5-HT terminals *via* the 5-HT transporter. DA has also been shown to increase the efflux of tritium in a concentration-dependent manner from rat neostriatal slices preloaded with [^{3}H]5-HT (Schmidt and Lovenberg, 1985). This DA-induced release was attenuated by 5-HT uptake inhibitors but not by DA uptake blockers, again providing evidence of active DA transport by the 5-HT carrier.

Faraj *et al.* (1994) have shown that the 5-HT uptake transporter is not specific for 5-HT but has relatively high affinity for DA. [^{3}H]DA uptake into human lymphocytes was 300 to 5000 times more potently inhibited by 5-HT uptake blockers than by DA or norepinephrine uptake inhibitors. In the present study, we demonstrated (fig. 5) that nearly 50% of the [^{3}H]DA uptake into hippocampal synaptosomes was sensitive to blockade by the selective 5-HT uptake inhibitor fluoxetine at a concentration well below that required for blockade of the DA uptake carrier (Wong *et al.*, 1974). The ability of fluoxetine to block MDMA-induced neurotoxicity up to 6 hr after MDMA treatment (Schmidt, 1987) would be consistent with the suggestion that protection at late times may be due to blockade of DA uptake by the 5-HT carrier.

The deamination of excessive DA by MAO-B within the 5-HT terminal could result in elevated levels of hydrogen peroxide, leading to membrane lipid peroxidation. Spina and Cohen (1989) have suggested a similar mechanism to explain the ability of an MAO inhibitor to block the increase in oxidized glutathione induced by accelerated DA turnover. Steranka and Rehind (1987) also reported that pretreatment with the free radical scavenger L-cysteine attenuated the long-term reduction in 5-HT and 5-HIAA levels observed in rats after PCA treatment, providing further support for a role of free radical formation in PCA- and MDMA-induced neurotoxicity. Recently, Gudelsky and Yamamoto (1994) have shown that MDMA increases striatal production of 2,3-dihydroxybenzoic acid from salicylate. This finding is consistent with the formation of hydroxy radicals after treatment with MDMA. MDA formation has been shown to be an indicator of lipid peroxidation (Ohkawa *et al.*, 1979), and in the present study we found that MDMA produced a significant increase in the formation of TBARS (fig. 3) that could be blocked by L-deprenyl (fig. 4).

The hippocampus has been shown to have very low levels of DA (Verney *et al.*, 1985). However, the possibility of volume transmission of DA from an area containing high concentrations of DA to the hippocampus may explain the selective serotonergic neurotoxicity seen in the hippocampus after treatment with MDMA (Schneider *et al.*, 1994). The deamination of excessive norepinephrine within the 5-HT terminals in the hippocampus may also be important in the selective 5-HT terminal degeneration seen after treatment with MDMA.

MDMA has been shown to induce a rapid decrease in TPH activity *in vivo* (Schmidt and Taylor, 1987). Stone *et al.* (1989) demonstrated that dithiothreitol and reduced iron could reverse MDMA-induced inhibition of TPH 3 hr after but not 18 hr after treatment with MDMA. These results

suggest that the acute decrease in TPH activity may result from oxidation of thiol groups within the TPH molecule. The present results clearly suggest a mechanism for generation of such an oxidative stress. In fact, L-deprenyl prevented the decrease in TPH activity seen 18 hr after MDMA treatment (fig. 6).

In contrast to the results of the present study, Benmansour and Brunswick (1994) have reported that L-deprenyl potentiated the toxicity of a low dose of PCA. These authors found that pretreatment of rats with L-deprenyl (1 mg/kg) 4 hr before PCA (2 mg/kg) resulted in a significant reduction in the number of [^{3}H]cyanoimipramine binding sites, as measured 7 days after PCA. In the same study, it was reported that this dose of PCA produced significant hypothermia 1 hr after treatment at an ambient temperature of 18°C. This finding is at odds with the results of Colado *et al.* (1993), who showed that PCA (2.5 mg/kg) caused no change in rectal temperature up to 2 hr after treatment. Although substituted amphetamines have been shown to both increase and decrease body temperature, decreases in body temperature are usually observed only when the ambient temperature is low (*e.g.*, 10°C; Gordon *et al.*, 1991). Martin *et al.* (1982) have shown that the nonspecific MAO inhibitor nialamide protects against the depletion of 5-HT seen after PCA treatment. In a preliminary study in our laboratory, a neurotoxic dose of PCA (10 mg/kg) resulted in a significant elevation of body temperature (+2.0°C) that was not blocked by 2 mg/kg of L-deprenyl (unpublished data). In our study, L-deprenyl appears to neither protect nor potentiate the decreases in B_{max} for [^{3}H] paroxetine binding 1 week after treatment with PCA (unpublished data). Further studies are under way in our laboratory to characterize the reasons for these different results. This may require considerable effort, however, because the doses, pretreatment times and ambient temperature in the study by Benmansour and Brunswick (1994) vary from those used in other laboratories.

We suggest that agents such as MDMA produce selective 5-HT terminal degeneration through the following sequence of events. (1) They cause the depletion of 5-HT from serotonergic neurons, which then renders the terminal vulnerable to the eventual toxic process. (2) They must dramatically increase DA synthesis and release; some of this increase may be attributable to the stimulation of 5-HT$_{2A}$ receptors through the release of neuronal 5-HT. (3) DA from this increased extracellular pool is then transported into the depleted 5-HT terminal by the 5-HT uptake carrier, where it is deaminated by MAO-B to generate hydrogen peroxide. (4) This hydrogen peroxide then leads to lipid peroxidation, perhaps other oxidative insults and selective 5-HT axonal degeneration. We believe this sequence of events is generally consistent with the results from numerous studies that have been conducted on the mechanism of the neurotoxicity of MDMA.

A more fundamental importance of these results concerns gaining a clearer picture of the reason for the localization of MAO-B within the 5-HT terminal. Although it has been speculated that it serves a protective role (Levitt *et al.*, 1982; Pintar *et al.*, 1983; Westlund *et al.*, 1985)—to degrade foreign transmitter molecules—it was only under the extreme conditions of amphetamine-induced toxicity that this function became evident. Under normal conditions, MAO-B deamination of extraneous DA that might occasionally enter the 5-HT terminal would be of little consequence, as the reductive capacity of the neuron could accommodate low levels of oxidants. However, treatment with MDMA may overwhelm the ability of the neuron to destroy oxidative species, leading to toxicity. Mechanisms similar to this may account for selective terminal and axonal degeneration produced by other substituted amphetamines. There may also be unidentified pathological states that result as a consequence of depletion of a particular neurotransmitter, followed by concentration of foreign transmitter molecules into the terminal. If the chemical defense mechanisms within the terminal are overwhelmed, the accumulation of metabolic products may lead to toxicity.

Finally, we believe these results should focus attention on the "classic" view of neuronal function. In traditional descriptions of neuron terminal function, the degradation of 5-HT to 5-HIAA occurs within serotonergic terminals, and the metabolism of DA to DOPAC occurs within dopaminergic terminals (which contain only MAO-A; Westlund *et al.*, 1985). However, the isozyme of MAO actually localized within the respective terminals has lower substrate specificity for the transmitter produced by that neuron than for that produced by the other. The results of the present study appear to be more consistent with earlier suggestions (Levitt *et al.*, 1982; Pintar *et al.*, 1983) that MAO serves a protective role within the neuron. This perspective appears to have great significance in gaining a better understanding of the role of MAO in various mood and behavioral states, its contribution to neurodegenerative processes and how and why specific inhibitors of MAO isozymes exert their therapeutic effects.

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