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Involvement of the serotonin transporter in the formation of hydroxyl radicals induced by 3,4-methylenedioxymethamphetamine

Mahalakshmi Shankaran [a], Bryan K. Yamamoto [b], Gary A. Gudelsky [a,*]

[a] *College of Pharmacy, University of Cincinnati, 3223 Eden Avenue, Cincinnati, OH 45267-0004, USA*
[b] *Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106, USA*

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Abstract

The mechanism of 3,4-methylenedioxymethamphetamine (MDMA)-induced depletion of brain serotonin (5-hydroxytryptamine, 5-HT) has been proposed to involve the generation of reactive oxygen species. In the present study, quantification of the extracellular concentration of 2,3-dihydroxybenzoic acid (2,3-DHBA) from salicylic acid was used as an index of hydroxyl radical generation. Although both MDMA and D-amphetamine markedly increased the extracellular concentration of dopamine in the striatum, only MDMA increased the extracellular concentration of 2,3-DHBA. Treatment with fluoxetine either 1 h prior to or 4 h following the administration of MDMA reduced the MDMA-induced formation of 2,3-DHBA and also attenuated the MDMA-induced depletion of 5-HT in the striatum. These results are supportive of the view that the MDMA-induced generation of hydroxyl radicals and, ultimately, the long-term depletion of 5-HT, is dependent, in part, on the activation of the 5-HT transporter.

Keywords: MDMA (3,4-methylenedioxymethamphetamine); 5-HT (5-hydroxytryptamine, serotonin); Hydroxyl radical; Neurotoxicity

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is considered to be potentially toxic to the serotonergic nerve terminals of experimental animals (Green et al., 1995; Sprague et al., 1998) and possibly humans (Bolla et al., 1998). MDMA produces biochemical and immunocytochemical effects such as a long-term depletion of brain serotonin (5-hydroxytryptamine, 5-HT) and its major metabolite 5-hydroxyindole acetic acid (Stone et al., 1986; Schmidt, 1987), a decrease in tryptophan hydroxylase activity (Schmidt and Taylor, 1987; Stone et al., 1987), a reduction in [^{3}H]paroxetine-labelled 5-HT uptake sites (Battaglia et al., 1987) and reduced immunostaining of 5-HT terminals (O'Hearn et al., 1988; Scallet et al., 1988).

Although the mechanism of MDMA-induced 5-HT toxicity has not yet been completely elucidated, there is evidence that psychostimulant-induced neurotoxicity is associated with the induction of oxidative stress. Gibb et al. (1990) suggested that MDMA promotes oxidative stress within 5-HT neurons on the basis of the observation that the MDMA-induced inactivation of tryptophan hydroxylase was reversed by sulfhydryl reducing conditions (Stone et al., 1988a). The promotion of a state of oxidative stress in 5-HT neurons by amphetamine analogues has been inferred from studies in which the administration of antioxidants or spin trap agents has been shown to prevent the 5-HT depletion produced by these psychostimulants (Steranka and Rhind, 1987; De Vito and Wagner, 1989; Schmidt and Kehne, 1990; Colado and Green, 1995; Gudelsky, 1996). In addition, Cadet et al. (1995) reported that transgenic mice that over-express the CuZn-superoxide dismutase gene are resistant to the neurotoxic effects of MDMA.

In further support of the view that the mechanism of MDMA-toxicity to 5-HT terminals involves oxidative stress is the fact that MDMA increases the formation of hydroxyl radicals, as evidenced by an increased conversion of salicylic acid to 2,3-dihydroxybenzoic acid (2,3-DHBA) (Gudelsky and Yamamoto, 1994; Colado et al., 1997, 1999a,b; Shankaran et al., 1999). Moreover, manipulations that attenuate the MDMA-induced formation of hydroxyl radicals are accompanied by protection against MDMA-in-

* Corresponding author. Tel.: +1-513-558-5735; fax: +1-513-558-4372.
E-mail address: gary.gudelsky@uc.edu (G.A. Gudelsky)

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duced 5-HT neurotoxicity (Colado et al., 1999a,b; Shankaran et al., 1999).

Factors that contribute to MDMA-induced hydroxyl radical formation and, ultimately, 5-HT neurotoxicity appear to include hyperthermia and dopamine. Drugs that diminish MDMA-induced hyperthermia also diminish the MDMA-induced generation of hydroxyl radicals and 5-HT neurotoxicity (Colado et al., 1999a,b). In addition, mazindol, a dopamine uptake inhibitor, attenuates the MDMA-induced increase in the extracellular concentration of dopamine, as well as the MDMA-induced generation of hydroxyl radicals and long-term depletion of striatal 5-HT (Shankaran et al., 1999).

There also is evidence that favors a role of the 5-HT transporter in the MDMA-induced depletion of brain 5-HT. The basis for this hypothesis is the finding that fluoxetine, a 5-HT transport inhibitor, attenuates MDMA-induced 5-HT neurotoxicity (Schmidt, 1987; Malberg et al., 1996; Aguirre et al., 1998). The finding that MDMA-induced hydroxyl radical formation is absent in rats in which 5-HT terminals have been depleted by fenfluramine (Colado et al., 1997) is additional support for the importance of the 5-HT terminal itself in the mechanism of MDMA-induced neurotoxicity.

In the present study, we sought to examine the role of the 5-HT transporter in the MDMA-induced generation of hydroxyl radicals and subsequent long-term depletion of striatal 5-HT with the use of fluoxetine.

2. Materials and methods

2.1. Animal procedures

Adult male rats (200–275 g) of the Sprague–Dawley strain (Charles River, Portage, MI) were used in the studies. The animals were housed three per cage in a temperature and light controlled room (lights on 0600–1800 h) until the day of surgery. All procedures were in strict adherence to the National Institutes of Health guidelines and approved by the Institutional Animal Care Committee.

2.2. In vivo microdialysis procedures

The formation of hydroxyl radicals was determined by quantifying the formation of 2,3-dihydroxybenzoic acid (2,3-DHBA) from salicylic acid (Floyd et al., 1984). For the experiments in which salicylic acid was perfused through the microdialysis probe, the rats were anesthetized with chloral hydrate (400 mg/kg, i.v.), and a microdialysis probe was implanted into the striatum (1.2 mm A, 3.1 mm L from bregma) according to the stereotaxic atlas of Paxinos and Watson (1986). The tip of the probe was 7 mm below the surface of the brain, and the probe was secured to the skull with screws and cranioplastic cement. The rats were allowed to recover, and the microdialysis experiment was performed the following day.

Concentric style microdialysis probes were constructed as described previously (Yamamoto and Pehek, 1990) with some modifications. The probes were constructed entirely from polyethylene and teflon tubing, in order to eliminate metal components, which promote the non-specific formation of hydroxyl radicals. The dialysis surface of the membrane (Spectra Por, 6000 MW cutoff, 210 μm outside diameter) for the striatum was 4.5 mm in length. The in vitro recoveries of the dialysis probes were 10–15% for dopamine and 2,3-DHBA, and no correction was made for recoveries.

On the day of the experiment, the probe was connected to an infusion pump set to deliver Dulbecco's phosphate buffered saline containing 1.2 mM $CaCl_2$ and 10 mM glucose at a rate of 1.8 ul/min. Following an equilibration period of 1.5 h, buffer including 5mM salicylic acid was perfused through the probe for the duration of the experiment. At least three baseline samples were obtained at 30-min intervals after which drug was administered, and samples were obtained thereafter at 1-h intervals.

2.3. Biochemical measurements

The extracellular concentration of 2,3-DHBA was quantified by high performance liquid chromatography (HPLC) with electrochemical detection. Aliquots (20 ul) of the dialysis samples were injected onto a C18 column (3 μm, 100×3 mm) connected to an electrochemical detector with a glassy carbon electrode maintained at 0.55 V relative to a Ag/AgCl reference electrode. The mobile phase was similar to that described by Althaus et al. (1995) and Fleckenstein et al. (1997), and was composed of 14.15 g/l monochloroacetic acid, 7% (vol./vol.) acetonitrile, 7% (vol./vol.) tetrahydrofuran, pH 2.4, pumped at a flow rate of 0.4 ml/min. Peak heights following injection of 20 μl samples were recorded with an integrator, and 2,3-DHBA content was calculated based on known standards. The retention time for 2,3-DHBA was approximately 9 min.

Dopamine in dialysis samples and 5-HT in striatal tissue were quantified with HPLC with electrochemical detection using methods similar to those described elsewhere (Gudelsky et al., 1994). Briefly, samples were injected onto a C18-column (5 μm, 100×2mm) connected to a LC-4B amperometric detector (Bioanalytical Systems, West Laffayette, IN). The mobile phase consisted of 35 mM citric acid, 54 mM sodium acetate, 50 mg/l of disodium ethylenediamine tetraacetate, 40 mg/l of octanesulfonic acid sodium salt, 4% (vol./vol.) methanol, pH 4.0, pumped at a flow rate of 0.4 ml/min. Peak heights following injection of 20 μl samples were recorded with an integrator, and the quantities of dopamine and 5-HT were calculated on the basis of known standards.

For postmortem analysis of brain 5-HT, the rats were killed by decapitation 7 days after the administration of

MDMA. The tissues were kept frozen (−80°C) until analyzed for 5-HT. The tissue samples were homogenized with 0.2 N perchloric acid and centrifuged for 6 min at 10,000 × *g*, and an aliquot of the resulting supernatant fluid was analyzed for 5-HT. The retention times for dopamine and 5-HT were approximately 6 and 18 min, respectively.

2.4. In vitro formation of hydroxyl radicals

Assessment of the in vitro formation of hydroxyl radicals was accomplished using a simple Fe^{2+}/ascorbate system based on the Fenton reaction and measuring the formation of 2,3-DHBA in the presence of salicylic acid. (Ste-Marie et al., 1996). The incubation mixture contained the following at final concentrations: 260 μl Dulbecco's phosphate buffer, 50 μl ferrous sulfate (1 mM), 170 ul ascorbic acid (100 μM), 20 μl disodium ethylenediamine tetraacetate (1 mM), 250 μl salicylic acid (1 mM), and 250 μl fluoxetine (0.1, 1, 3 or10 μM). In the blank solution, fluoxetine was replaced with water (250 μl). The amount of 2,3-DHBA formed was measured at 0, 20, 40 and 60 min by the HPLC method described above.

2.5. Drugs and drug treatments

The racemic mixture of MDMA hydrochloride was provided by the National Institute on Drug Abuse. MDMA was dissolved in 0.15 M NaCl and injected s.c. in a volume of 1 ml/kg. D-Amphetamine (Sigma, St. Louis, MO) was dissolved in 0.15 M NaCl and administered i.p. in a volume of 1 ml/kg. Fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN) was dissolved in 0.15 M NaCl and administered i.p. in a volume of 1 ml/kg, and injected 1 h prior to or 4 h prior after the injection of MDMA.

2.6. Statistical analysis

Analysis of the tissue concentrations of 5-HT was performed using a one-way analysis of variance, and differences between treatment groups were assessed with the Student–Newman–Keuls test. Data from dialysis experiments were analyzed using a two-way repeated measures analysis of variance (Sigmastat, Jandel Scientific). Multiple pairwise comparisons were performed using the Student–Newman–Keuls test. The rate of formation of 2,3-DHBA in vitro was assessed by linear regression analysis, and the comparison of slopes was done by one-way analysis of variance. Treatment differences were considered statistically significant at $P < 0.05$.

3. Results

The generation of hydroxyl radicals was estimated from the production of 2,3-DHBA during the perfusion of the dialysis probe with salicylic acid (5 mM). A single administration of MDMA (20 mg/kg, s.c.) significantly increased the extracellular concentration of 2,3-DHBA in the striatum when compared to values for vehicle-treated animals [main drug effect, $F(2, 18) = 15.08$, $P < 0.0001$] (Fig. 1A). However, the extracellular concentration of 2,3-DHBA in the striatum was not significantly affected by D-amphetamine (10 mg/kg, i.p.). Both MDMA (20 mg/kg, s.c.) and D-amphetamine (10 mg/kg, i.p.) significantly increased the extracellular concentration of dopamine when compared to vehicle-treated controls [main drug effect, $F(2, 18) = 11.8$, $P = 0.0005$] (Fig. 1B), and the maximal increase elicited by D-amphetamine was significantly greater than that produced by MDMA [drug × interval, $F(16, 144) = 21.4$, $P < 0.0001$].

The effect of fluoxetine (10 mg/kg, i.p.) on the MDMA-induced increase in the extracellular concentration of 2,3-DHBA is shown in Fig. 2A. In rats treated with fluoxetine 1 h prior to the injection of MDMA, the extracellular concentration of 2,3-DHBA was significantly less than that in rats treated only with MDMA [main drug effect, $F(3, 23) = 11.47$, $P < 0.0001$). The administration of fluoxetine also significantly attenuated the magnitude of the MDMA-induced increase in the extracellular concen-

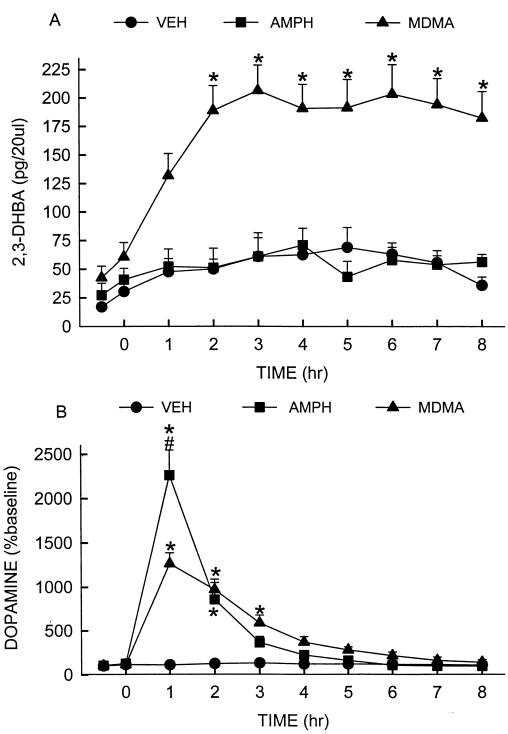

Fig. 1. Effect of MDMA and D-amphetamine on the extracellular concentration of 2,3-DHBA (A) and dopamine (B) in the striatum of rats. MDMA (20 mg/kg, s.c.) or D-amphetamine (10 mg/kg, i.p.) was injected at time 0. Perfusion with buffer containing 5 mM salicylic acid commenced 1.5 h before time 0 and continued throughout the experiment. The values represent the mean ± standard error of 6–7 rats. * Indicates values that differ significantly ($p < 0.05$) from those of the vehicle (VEH)-treated animals.

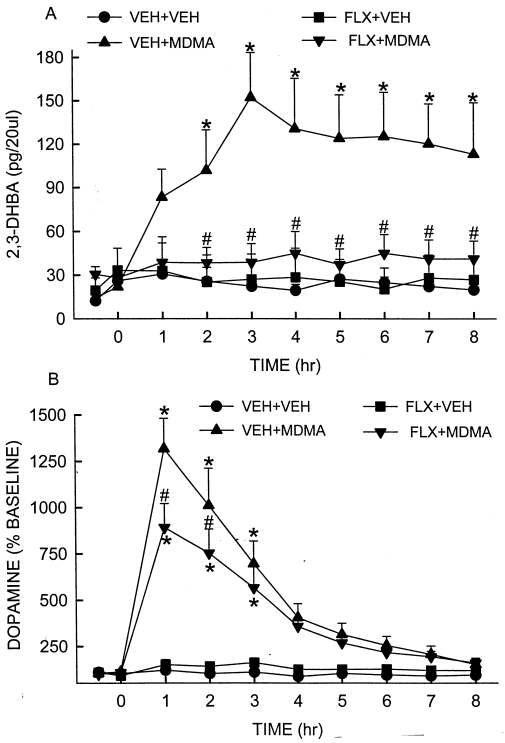

Fig. 2. Effect of MDMA on the extracellular concentration of 2,3-DHBA (A) and dopamine (B) in the striatum of rats treated with fluoxetine. MDMA (20 mg/kg, s.c.) was injected at time 0. Fluoxetine (10 mg/kg, i.p.) was injected 1 h before the MDMA injection. Perfusion with buffer containing 5mM salicylic acid commenced 1.5 h before time 0 and continued throughout the experiment. The values represent the mean ± standard error of 6–8 rats. * Indicates values that differ significantly ($p < 0.05$) from those of the vehicle (VEH)-treated animals. # Indicates values that differ significantly ($p < 0.05$) from those of MDMA-treated animals.

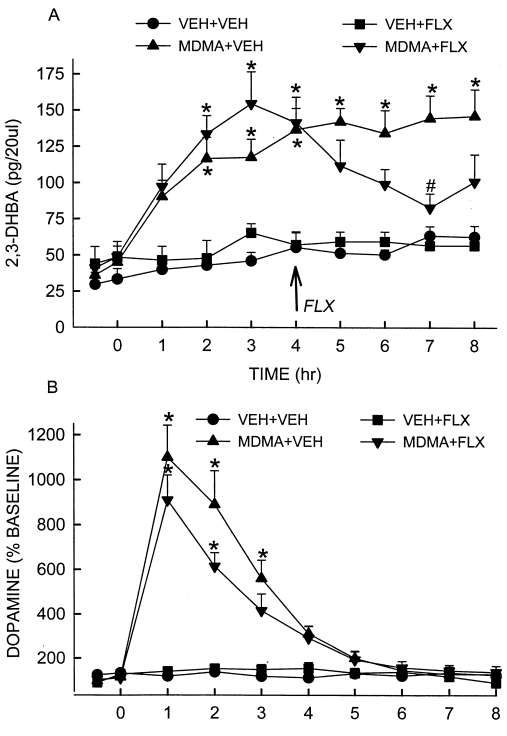

Fig. 3. Effect of fluoxetine on the MDMA-induced increase in the extracellular concentration of 2,3-DHBA (A) and dopamine (B) in the striatum. MDMA (20 mg/kg, s.c.) was injected at time 0. Fluoxetine (10 mg/kg, i.p.) was injected 4 h after the MDMA injection. Perfusion with buffer containing 5mM salicylic acid commenced 1.5 h before time 0 and continued throughout the experiment. The values represent the mean ± standard error of 6–8 rats. * Indicates values that differ significantly ($p < 0.05$) from those of the vehicle (VEH)-treated animals. # Indicates values that differ significantly ($p < 0.05$) from those of MDMA-treated animals.

tration of dopamine in the striatum [drug × interval, $F(24, 184) = 12.9$, $P < 0.0001$] (Fig. 2B).

In order to obviate the concern that a diminished dopamine response to MDMA in rats treated with fluoxetine might account for the diminished extracellular concentration of 2,3-DHBA, rats were treated with fluoxetine 4 h after the administration of MDMA. The administration of fluoxetine 4 h after treatment with MDMA did not alter the time course for the elevation of the extracellular concentration of dopamine (Fig. 3B). Nevertheless, the administration of fluoxetine at this time did result in a significant reduction in the elevation of the extracellular concentration of 2,3-DHBA elicited by MDMA [main drug effect, $F(3,22) = 11.85$, $P < 0.0001$] (Fig. 3A).

The effect of fluoxetine on the generation of hydroxyl radicals under in vitro conditions was also ascertained. Incubation of 1 mM salicylic acid with a Fe^{2+}/ascorbic acid system resulted in an increase in the formation of 2,3-DHBA over the 60-min period. The in vitro generation of 2,3-DHBA was not significantly affected by fluoxetine in concentrations of 0.1–3 μM. However, the generation of 2,3-DHBA was significantly suppressed by approximately 45% in the presence of 10 μM fluoxetine (Fig. 4).

The effects of MDMA, D-amphetamine and fluoxetine on the tissue concentration of 5-HT in the striatum are

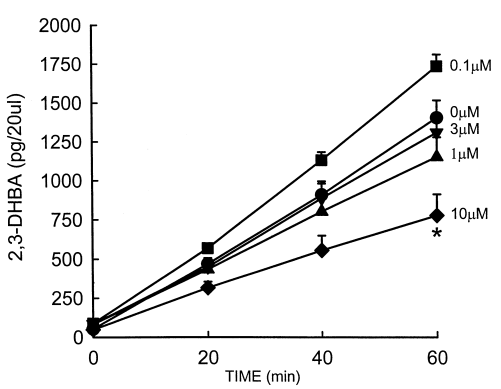

Fig. 4. Effect of fluoxetine on the rate of formation of 2,3-DHBA in vitro. Fluoxetine at concentrations of 0.1, 1, 3, and 10 μM were added to the incubation mixture at time 0. The values represent the mean ± S.E. of 6 repetitions for each concentration of fluoxetine. * Indicates that the slope of the line differs significantly ($p < 0.05$) from that of the line representing 0 μM fluoxetine.

Table 1
Tissue concentration of 5-HT in the striatum one week following drug administration
In three separate experiments, animals were injected with vehicle (VEH), MDMA (20 mg/kg, s.c.) or D-amphetamine (AMPH, 10 mg/kg, i.p.). Fluoxetine was injected 1 h before (Fluoxetine + MDMA) or 4 h after (MDMA + Fluoxetine) the administration of MDMA. All the animals were killed 7 days after drug administration. Since there were no differences in the values for either the vehicle (VEH)-treated rats or the MDMA-treated rats between the different experiments, they have been combined for ease of presentation. However, statistical analyses were performed on data from each experiment.
N = number of rats

DRUG	N	5-HT (ng/mg tissue)
VEH	20	0.50 ± 0.02
MDMA	23	0.35 ± 0.01[a]
AMPH	7	0.49 ± 0.03
Fluoxetine	11	0.48 ± 0.02
Fluoxetine + MDMA	7	0.44 ± 0.03
MDMA + Fluoxetine	7	0.43 ± 0.03

[a] Indicates values that were significantly different from VEH-treated animals.

shown in Table 1. Whereas the striatal concentration of 5-HT was unaffected 7 days following a single injection of D-amphetamine (10 mg/kg, i.p.), an injection of MDMA (20 mg/kg, s.c.) resulted in a 30% ($p < 0.05$) reduction in the striatal concentration of 5-HT. The administration of fluoxetine significantly attenuated the MDMA-induced 5-HT depletion in the striatum, regardless of whether fluoxetine was given 1 h before or 4 h after the administration of MDMA.

4. Discussion

Although the exact mechanism of MDMA-induced 5-HT toxicity is unknown, there is increasing evidence in support of the hypothesis that oxidative stress due to the formation of free radicals may be involved in MDMA-induced damage to 5-HT terminals. The findings that free radical scavengers and antioxidants attenuate the MDMA-induced depletion of 5-HT (Schmidt and Kehne, 1990; Colado and Green, 1995; Gudelsky, 1996) provide indirect evidence for the involvement of free radicals in the mechanism of MDMA neurotoxicity. In addition, MDMA has been reported to produce cellular changes, e.g., lipid peroxidation, protein nitration, consistent with the formation of free radicals (Sprague and Nichols, 1995; Colado et al., 1997; Yamamoto and Gudelsky, unpublished observations). Finally, Green and co-workers, as well as Gudelsky and colleagues, have demonstrated that MDMA increases the formation of hydroxyl radicals, as evidenced by an increased extracellular concentration of 2,3-DHBA following salicylic acid administration (Colado et al., 1997 1999a,b; Gudelsky and Yamamoto, 1994; Shankaran et al., 1999). The stimulatory effect of MDMA on the formation of 2,3-dihydroxybenzoic acid reported in the present study is consistent with these previous reports.

The activation of the 5-HT transporter by MDMA appears to exert a critical role in the long-term toxicity to 5-HT terminals. In accord with previous studies (Schmidt, 1987; Malberg et al., 1996; Aguirre et al., 1998), treatment of rats with fluoxetine provided protection against the MDMA-induced depletion of 5-HT in the striatum. It is additionally noteworthy that the administration of fluoxetine 4 h following the administration of MDMA afforded neuroprotection against MDMA-induced 5-HT toxicity. Schmidt (1987) also has reported that treatment of rats with fluoxetine up to 6 h after an injection of MDMA significantly attenuates MDMA-induced 5-HT neurotoxicity.

The administration of fluoxetine either prior to or following the administration of MDMA resulted in a reduction of the MDMA-induced formation of hydroxyl radicals. It is unlikely that fluoxetine directly scavenges hydroxyl radicals, inasmuch as the in vitro generation of hydroxyl radicals was not affected by fluoxetine in the present study at concentrations that are normally attained in the brain (Caccia et al., 1992; Gardier et al., 1993; Malagie et al., 1996). It also is unlikely that fluoxetine prevents the MDMA-induced formation of hydroxyl radicals through an attenuation of MDMA-induced hyperthermia. Although pharmacological agents that suppress MDMA-induced hyperthermia attenuate hydroxyl radical formation (Colado et al., 1999a,b), fluoxetine has been shown not to diminish the hyperthermic response to MDMA (Malberg et al., 1996; Aguirre et al., 1998).

One potential mechanism by which fluoxetine attenuates the MDMA-induced formation of hydroxyl radicals is by preventing the entry into the 5-HT terminal of reactive substances that are capable of generating free radicals. The importance of the 5-HT terminal itself for the generation of hydroxyl radicals is underscored by the finding that MDMA-induced hydroxyl radical formation is absent in rats in which 5-HT terminals have been disrupted by fenfluramine (Colado et al., 1997). Potential sources of hydroxyl radicals within the 5-HT terminal include the oxidation of dopamine or MDMA itself (Graham, 1978; Schmidt and Lovenberg, 1985; Cohen, 1987; Hiramatsu et al., 1990; Faraj et al., 1994; Colado et al., 1995).

It can be envisioned that excessive extracellular dopamine may give rise to the formation of reactive oxygen species (c.f., Cadet and Brannock, 1998), and the MDMA-induced increase in hydroxyl radical formation may simply be a consequence of the increase in the extracellular concentration of dopamine. However, in the present study, unlike MDMA, D-amphetamine, which also produced an immediate and large increase in the extracellular concentration of dopamine, did not enhance the formation of hydroxyl radicals. Huang et al. (1997) also have reported that the administration of amphetamine alone

does not increase hydroxyl radical formation. These results are suggestive that the generation of hydroxyl radicals is not simply the result of an increase in the extracellular concentration of dopamine.

Nevertheless, there is recent evidence for a role of dopamine in the MDMA-induced generation of hydroxyl radicals, as well as the long-term depletion of striatal 5-HT (Stone et al., 1988b; Brodkin et al., 1993, Shankaran et al., 1999). On the basis of the present results, it is tempting to suggest that dopamine is necessary, but not sufficient, to allow for the MDMA-induced generation of hydroxyl radicals and 5-HT toxicity. This view is supported by the finding that the combined administration of amphetamine with the non-neurotoxic MDMA analog, 5-methoxy-6-methyl-2-aminoindan, which produces carrier-mediated 5-HT release, results in 5-HT neurotoxicity, although neither of these drugs produces damage to 5-HT terminals when administered alone (Johnson and Nichols, 1991). These findings further strengthen the view that activation of the 5-HT transporter by MDMA is essential for producing damage to 5-HT terminals. Sprague et al. (1998) have speculated that dopamine released by MDMA may enter the 5-HT terminal through an activated 5-HT transporter and be oxidized by monoamine oxidase-B enzyme present within the 5-HT terminal leading to the generation of free radicals.

However, it is difficult to reconcile the postulated role of dopamine in MDMA-induced hydroxyl radical formation and 5-HT toxicity in brain regions, e.g., hippocampus, which receive sparse dopaminergic innervation. Indeed, the role of dopamine in MDMA-induced 5-HT toxicity in the hippocampus (Shankaran and Gudelsky, 1998) and hydroxyl radical formation in other brain regions (Colado et al., 1999b) is somewhat controversial. The finding that hydroxyl radical formation was reduced in MDMA-treated rats given fluoxetine at a time, viz., 4 h after MDMA treatment, when extracellular concentrations of dopamine had returned to baseline values also appears to be inconsistent with the view that dopamine oxidation is a source of hydroxyl radicals.

An alternative source of hydroxyl radicals may be MDMA itself. The metabolism of MDMA produces catechols and quinones that can result in the further formation of free radicals (Hiramatsu et al., 1990; Colado et al., 1995). Moreover, MDMA produces a massive release of 5-HT (Gudelsky and Nash, 1996) which can be acted upon by hydroxyl radicals to generate species that may be toxic to 5-HT terminals (Wong et al., 1993; Wrona and Dryhurst, 1998). It is noteworthy that the concentration of MDMA in the brain 4 h after its administration is substantial and is approximately the same as that attained 30 min after its administration (Gudelsky, 1996). The ability of fluoxetine treatment 4 h after the administration of MDMA to reduce hydroxyl radical formation might be indicative of a continued action of MDMA itself through the 5-HT transporter to generate hydroxyl radicals.

In summary, the results of the present study lend additional support to the view that MDMA increases the formation of hydroxyl radicals. Moreover, beyond the potential roles of hyperthermia (Colado et al., 1999a,b) and the dopamine transporter (Shankaran et al., 1999) in the process of MDMA-induced hydroxyl radical formation, the present results also implicate the 5-HT transporter in the mechanism of hydroxyl radical formation and 5-HT neurotoxicity produced by MDMA. However, the identity of the species that ultimately gives rise to hydroxyl radical formation remains to be determined. Nevertheless, the findings that drugs (e.g., mazindol, fluoxetine, chlormethiazole) that suppress the MDMA-induced formation of hydroxyl radicals also suppress MDMA-induced 5-HT neurotoxicity provide additional support for the view that the mechanism of MDMA-induced neurotoxicity to 5-HT terminals involves the induction of oxidative stress following the increased formation of hydroxyl radicals.

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