L-Tyrosine Contributes to (+)-3,4-Methylenedioxymethamphetamine-Induced Serotonin Depletions

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The specific mechanisms underlying (+)-3,4-methylenedioxymethamphetamine (MDMA)-induced damage to 5-HT terminals are unknown. Despite the hypothesized role for dopamine (DA) and DA-derived free radicals in mediating this damage, it remains unclear why MDMA produces long-term depletions of 5-HT in brain regions that are sparsely innervated by DA neurons. We hypothesized that the precursor to DA biosynthesis, tyrosine, mediates MDMA-induced 5-HT depletions. Extracellular tyrosine concentrations increased fivefold in striatum and 2.5-fold in hippocampus during the administration of neurotoxic doses of MDMA. *In vitro* results show that L-tyrosine can be hydroxylated nonenzymatically to the DA precursor L-3,4-dihydroxyphenylalanine (DOPA) under pro-oxidant conditions. The local infusion of L-tyrosine into the striatum or hippocampus during MDMA administration potentiated the acute increase in extracellular DA and the long-term depletion of 5-HT after MDMA. Coinfusion of the aromatic amino acid decarboxylase (AADC) inhibitor *m*-hydroxybenzylhydrazine attenuated these effects in hippocampus and decreased basal extracellular DA in the striatum. In contrast, the reverse dialysis of the tyrosine hydroxylase inhibitor α -methyl-*p*-tyrosine into the hippocampus did not affect MDMA-induced increases in extracellular DA or the long-term depletion in 5-HT. These results show that MDMA increases the concentration of tyrosine in the brain to cause a long-term depletion of 5-HT via the nonenzymatic, tyrosine hydroxylase-independent, hydroxylation of tyrosine to DOPA and subsequently to DA via AADC. Overall, the findings suggest that MDMA depletes 5-HT by increasing tyrosine and its eventual conversion to DA within 5-HT terminals.

Key words: dopamine; tyrosine; MDMA; striatum; hippocampus; serotonin

Introduction

(+)-3,4-Methylenedioxymethamphetamine (MDMA) (known as “ecstasy,” “E,” or “X”) is an abused amphetamine derivative (McKenna and Peroutka, 1990). MDMA selectively damages serotonin (5-HT) neurons in rats and nonhuman primates as evidenced by reduced brain concentrations of 5-HT, loss of tryptophan hydroxylase activity (Stone et al., 1986; Battaglia et al., 1987; Schmidt and Taylor, 1987), and reductions in 5-HT uptake sites in striatum, hippocampus, and prefrontal cortex (Ricaurte, 1989; Slikker et al., 1989; Battaglia et al., 1991; Scheffel et al., 1998).

Although the specific mechanisms underlying MDMA-induced neurotoxicity are unknown, oxidative stress is a factor. MDMA increases hydroxyl radical production (Gudelsky and Yamamoto, 1994; Colado and Green, 1995; Shankaran et al., 1999), and antioxidants protect against MDMA-induced 5-HT depletions (Zheng and Laverty, 1998; Shankaran et al., 2001).

One source of free radicals is the metabolism of dopamine (DA) by monoamine oxidase-B (MAO-B) to produce H_2O_2 that is reduced by iron to produce hydroxyl radicals (Cohen, 1987). MDMA increases DA release (Yamamoto and Spanos, 1988), and the DA precursor L-3,4-dihydroxyphenylalanine (DOPA) exacerbates MDMA-induced 5-HT depletions (Schmidt et al., 1991). Conversely, inhibition of DA synthesis (Stone et al., 1988), depletion of DA stores with reserpine (Stone et al., 1988), destruction of DA neurons (Schmidt et al., 1990), inhibition of MAO-B (Sprague and Nichols, 1995), and gene knockout of MAO-B (Falk et al., 2002) all prevent MDMA-induced damage to 5-HT neurons. It should be noted that MDMA-induced damage to 5-HT neurons is dependent on induction of hyperthermia (Broening et al., 1995), and catecholaminergic drugs that decrease body temperature may alter MDMA-induced toxicity in a manner independent of toxicity produced by DA.

Sprague et al. (1998) proposed that DA is heterologously transported into 5-HT terminals by the 5-HT transporter to mediate MDMA-induced 5-HT depletions. However, the origin of DA and why MDMA-induced 5-HT depletions occur in the DA-sparse hippocampus (Versteeg et al., 1976; Diop et al., 1988) is unclear. Furthermore, MDMA administered directly into the brain causes DA release but not long-term 5-HT depletions (Nixdorf et al., 2001). Moreover, prevention of MDMA-induced neu-

Received Aug. 10, 2005; revised Nov. 9, 2005; accepted Nov. 10, 2005.

This work was supported by National Institutes of Health Grants DA16866, DA16486, and DA19486 and by Hitachi America.

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DOI:10.1523/JNEUROSCI.3353-05.2006

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rotoxicity by catecholaminergic drugs (e.g., reserpine) may be attributable to blockade of MDMA-induced hyperthermia and unrelated to the attenuation of DA transmission (Yuan et al., 2002).

Although DA most likely contributes to 5-HT terminal damage via uptake into 5-HT terminals in areas with preexisting DAergic innervation, it cannot explain severe 5-HT loss in DA-sparse regions such as hippocampus. Therefore, another source of DA must contribute to the long-term MDMA-induced 5-HT depletions. One possibility is the *de novo* synthesis of DA from tyrosine. Tyrosine is transported into terminals for DA synthesis. Because MDMA produces hydroxyl radicals, tyrosine might be nonenzymatically hydroxylated to DOPA within 5-HT terminals and eventually to DA by aromatic amino acid decarboxylase (AADC) endogenous to 5-HT neurons.

To examine whether tyrosine contributes to MDMA-induced toxicity to 5-HT terminals in the DA-rich striatum and the DA-sparse hippocampus, we measured brain tyrosine after MDMA, the nonenzymatic hydroxylation of tyrosine to DOPA by hydroxyl radicals, and the effects of tyrosine on extracellular DA and MDMA-induced depletions of 5-HT. We also examined whether inhibition of tyrosine hydroxylase (TH) or AADC alters extracellular DA and blocks the toxic effects of tyrosine and/or MDMA on 5-HT terminals.

Materials and Methods

Subjects

Adult male Sprague Dawley rats (200–250 g, Harlan Sprague Dawley, Indianapolis, IN) were maintained on a 12 h light/dark cycle (lights from 7:00 A.M. to 7:00 P.M.). Rats were group housed until surgery and then singly housed in a temperature- and humidity-controlled environment. Food and water were available *ad libitum*. All experiments and procedures were conducted in strict adherence to guidelines set by the National Institutes of Health and approved by the Institutional Animal Care and Use Committee.

Drugs and reagents

(+)-MDMA hydrochloride was generously provided by the National Institute on Drug Abuse (Rockville, MD). L-Tyrosine was purchased from Calbiochem (La Jolla, CA), and L-valine, Dulbecco's PBS, *m*-hydroxybenzylhydrazine (NSD 1015), α -methyl-DL-*p*-tyrosine (α MPT), *o*-phthalaldehyde (OPA), L-ascorbic acid, ferrous ammonium sulfate, and EDTA were purchased from Sigma-Aldrich (St. Louis, MO).

MDMA was dissolved in 0.9% saline or Dulbecco's PBS for intraperitoneal injections and reverse dialysis, respectively.

Experimental design

Experiment 1: systemic versus local perfusion of MDMA and extracellular tyrosine concentrations. To determine whether MDMA increases the extracellular concentration of tyrosine in the striatum and hippocampus, rats were administered neurotoxic doses of MDMA (intraperitoneally, a total of four injections of 10 mg/kg, each injection every 2 h for 6 h) or 0.9% saline (intraperitoneally, a total of four injections of 1 ml/kg, each injection every 2 h for 6 h). Extracellular concentrations of tyrosine were determined via *in vivo* microdialysis and HPLC coupled with electrochemical detection (HPLC-EC) (for details, see below). One week after the dialysis experiments, rats were killed, and their brains were removed and frozen. The striatum and hippocampus were dissected and assayed for DA and 5-HT by HPLC-EC. The striatum and hippocampus were examined to represent commonly examined areas that are depleted of 5-HT after MDMA but differ in their innervation by DA terminals.

In separate experiments, MDMA (100 μ M) or Dulbecco's alone [artificial CSF (aCSF)] was locally perfused into the lateral striatum in rats during dual-probe microdialysis experiments (for details, see below) to determine the effects of the local administration of MDMA on the extracellular concentrations of tyrosine. The concentration of MDMA used in

these experiments was based on previously published work in this laboratory (Nixdorf et al., 2001) indicating that 100 μ M MDMA produces an increase in extracellular DA concentrations similar to that seen with neurotoxic doses of MDMA (Nash and Yamamoto, 1992) and that brain MDMA concentrations are $\sim$ 250 μ M after a single 40 mg/kg dose (Chu et al., 1996). Tyrosine concentrations were determined by HPLC-EC.

One week after the dialysis experiment, the rats were killed, their brains were frozen, and 400- μ m-thick coronal sections were obtained through the striatum and hippocampus. The tissue within 1 mm of the area surrounding the dialysis probe tracts was dissected under a microscope and subsequently assayed for DA and 5-HT.

Experiment 2: systemic versus local MDMA administration and reverse dialysis of L-tyrosine and L-valine. MDMA or saline was administered in the concentrations and doses mentioned above for experiment 1. L-Tyrosine (500 μ M) and L-Valine (500 μ M) were reverse dialyzed into one striatum on either side of the same rat (e.g., L-tyrosine through one probe and L-valine through the other) from the beginning of dialysis perfusion and throughout the experiment to determine their ability to alter MDMA-induced 5-HT depletions. The rationale for the choice of concentration of L-tyrosine was based on the fivefold increase in tyrosine observed in the striatum in Figure 1, the estimation of a basal brain tyrosine concentration of 10 μ M based on literature values (Reichel et al., 1995; Le Masurier et al., 2005), and preliminary studies indicating that the reverse dialysis and *in vitro* recovery of tyrosine by the dialysis probes was $\sim$ 10%. Thus, 500 μ M L-tyrosine (10 times at 50 μ M) was reverse dialyzed. In the present study, the basal brain tyrosine concentration across all groups was determined to be 12.71 ± 0.86 μ M, within the reported range of 8–19 μ M (Reichel et al., 1995; Le Masurier et al., 2005). L-Valine (500 μ M) served as a control for the concentration of large neutral amino acid (LNAA) perfused locally. One week later, the tissue within 1 mm of the area surrounding the dialysis probe tracts was dissected under a microscope and subsequently assayed for DA and 5-HT.

In separate experiments, MDMA (100 μ M) alone or in combination with L-tyrosine (500 μ M) or L-valine (500 μ M) was reverse dialyzed into the striatum to determine whether increasing local striatal tyrosine concentrations can produce a 5-HT depletion with the local administration of MDMA. One week later, the tissue within 1 mm of the area surrounding the dialysis probe tracts was dissected under a microscope and subsequently assayed for DA and 5-HT.

Experiment 3: *in vitro* oxidation of L-tyrosine to DOPA. Various concentrations of L-tyrosine (25, 62.5, 125, and 250 μ M; $n = 6$ per concentration) were added to an *in vitro* hydroxyl radical-generating system (for details, see below) (Ste-Marie et al., 1996) at room temperature to determine whether DOPA can be formed from tyrosine in a pro-oxidant environment. The advantage of this *in vitro* preparation is that the nonenzymatic hydroxylation of L-tyrosine specifically can be tested in the absence of tyrosine hydroxylase or tyrosinase under controlled pro-oxidant conditions. The range of tyrosine concentrations used in this assay was based on three factors: (1) basal brain tyrosine concentrations of $\sim$ 7–10 μ M (Reichel et al., 1995), (2) the *in vitro* recovery and reverse dialysis of tyrosine by the microdialysis probe to be $\sim$ 10–18%, and (3) that MDMA produces a fivefold increase in extracellular tyrosine concentrations (see Fig. 1). Collectively, these factors result in a brain tyrosine concentration of $\sim$ 50 μ M after MDMA. Therefore, concentrations above and below the estimated 50 μ M tyrosine achieved in the striatum after systemic administration of MDMA were chosen for the production of DOPA *in vitro*. Twenty microliter aliquots were collected at 5, 15, 30, 45, 60, and 120 min after the addition of tyrosine to the reaction mixture. DOPA was determined by HPLC-EC.

Experiment 4: systemic versus local MDMA administration and manipulation of tyrosine and dopamine in striatum and hippocampus. The neurotoxic regimen of MDMA or saline was administered systemically, and all possible combinations of L-tyrosine (500 μ M), the AADC inhibitor NSD 1015 (100 μ M), and aCSF alone (e.g., L-tyrosine alone through one probe or L-tyrosine in combination with NSD 1015 through the other probe implanted in the same brain region on the contralateral side) were administered bilaterally by reverse dialysis during dual-probe microdialysis experiments in the striatum or hippocampus throughout the entire course of the dialysis experiment. Extracellular concentrations of DA

were determined by HPLC-EC. Rats were killed 1 week after the microdialysis experiment, and the tissue within 1 mm of the area surrounding the dialysis probe tracts was dissected under a microscope and subsequently assayed for 5-HT. The concentration of NSD 1015 used was chosen based on previous work showing that 100 μM effectively inhibited AADC and increased DOPA accumulation in the nucleus accumbens (Brock et al., 1990).

In other dual-probe microdialysis experiments, the tyrosine hydroxylase inhibitor α -methyl-*p*-tyrosine (αMPT) (100 μM) or aCSF alone was reverse dialyzed into the ventral hippocampus during the neurotoxic regimen of MDMA or saline. αMPT was locally perfused into the hippocampus to avoid the known hypothermic effect seen with systemic administration after MDMA (Yuan et al., 2002). The concentration of αMPT used was based on the demonstration that 100 μM αMPT significantly decreased basal DA release in the nucleus accumbens (Tuinstra and Cools, 2000) and that the K_m of tyrosine for tyrosine hydroxylase ranges from 20 to 55 μM (Kuczenski and Mandell, 1972; Bakht et al., 1980; Lazar et al., 1982).

In separate dual-probe microdialysis experiments, MDMA was coinfused locally in the striatum with either L-tyrosine (500 μM) alone or the combination of L-tyrosine and NSD 1015 (100 μM). One week later, rats were killed, and the tissue within 1 mm of the area surrounding the dialysis probe tracts was dissected under a microscope and subsequently assayed for DA and 5-HT.

General methods

Surgical procedures. Rats were anesthetized with ketamine hydrochloride (70 mg/kg, i.m.) and xylazine (7 mg/kg, i.m.). The skull was exposed, and holes were drilled over the left and right striatum (1.2 mm anterior, 3.2 mm lateral from bregma) or the left and right ventral hippocampus (5.8 mm posterior, 5.25 mm lateral from bregma) (Paxinos and Watson, 1986). Guide cannulas (21 gauge) were positioned onto the dura within each hole and cemented to the skull with cranioplastic cement and three stainless steel machine screws. Obturators were constructed from 31 gauge stainless steel wire and inserted immediately after surgery through the guide cannulas so that they terminated flush with the end of the guide cannulas. Rats were allowed to recover for 3 d before the dialysis experiment.

In vivo microdialysis. Concentric microdialysis probes were constructed as described previously (Yamamoto and Pehek, 1990). The length of the dialysis membrane (Spectra/Por; Spectrum Laboratories, Rancho Dominguez, CA) (13,000 molecular weight cutoff; 210 μm outer diameter) was 4 mm for both the striatum and ventral hippocampus placements. Before probe insertion, a 26 gauge stainless steel needle with a beveled tip was inserted into each guide cannula to extend 0.5 mm beyond the guide cannula to puncture the dura. Probes were inserted slowly through guide cannulas into the awake rat 18 h before the start of the microdialysis experiment. The probes were connected to a model 22 syringe perfusion pump (Harvard Apparatus, Holliston, MA) via polyethylene 50 (PE50) tubing and a two-channel liquid swivel (Instech, Plymouth Meeting, PA). A spring tether covered the PE50 tubing and connected the rat to a swivel that allowed for relatively unrestrained movement. Dialysis perfusion using a modified Dulbecco's PBS (in mM: 138 NaCl, 2.7 KCl, 0.5 MgCl_2 , 1.5 KH_2PO_4 , 8.1 Na_2HPO_4 , 1.2 CaCl_2 , and 5.0 glucose, pH 7.4) alone or in combination with other drugs or reagents was initiated 18 h after probe insertion. Perfusion flow rate was 1.0 $\mu\text{l}/\text{min}$. Dialysate was collected in microcentrifuge tubes via microbore tubing. Two hours were allowed for a prebaseline equilibration period before the collection of baseline samples. Baseline samples were collected hourly for 2 h before injection of MDMA or saline. Drugs that were reversed dialyzed into the striatum began with the onset of perfusion (e.g., beginning of prebaseline) 2 h before the collection of baseline samples.

In vitro formation of hydroxyl radicals. Hydroxyl radicals were produced using a Fe^{2+} /ascorbate system based on the Fenton reaction (Ste-Marie et al., 1996). The reaction mixture consisted of 50 μM ferrous ammonium sulfate, 17 μM ascorbic acid, 20 μM EDTA, 510 μl Dulbecco's PBS, and 250 μl of various concentrations of tyrosine. Tyrosine was added in the final concentrations of 25, 62.5, 125, and 250 μM . The total

reaction volume was 1 ml. Each reaction was conducted at room temperature.

Analysis of tyrosine, DOPA, and DA. Extracellular concentrations of DA were measured as described previously (Donzanti and Yamamoto, 1988; Yamamoto and Davy, 1992). Twenty microliter aliquots were used for assay of DA by HPLC-EC (Nash and Yamamoto, 1992). Separation from metabolites was achieved with a reverse-phase column (Phenomenex, Belmont, CA) (C-18 column; 3 μm particle size; 100 $\times$ 2 mm) and a mobile phase consisting of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM Na_2EDTA , 0.22 mM octyl sodium sulfate, and 3% methanol, pH 4.2. Measurement of DOPA was accomplished with the same assay parameters except that the pH was decreased to 2.5 to separate DOPA from the solvent front. Extracellular tyrosine concentrations were analyzed using 20 μl aliquots by HPLC-EC after precolumn derivatization with OPA and sodium sulfite as described previously (Bongiovanni et al., 2001). The OPA derivatizing agent (10 μl) was added to each sample by an autoinjector, mixed, and allowed to react for 2 min before injecting onto the HPLC column (C-18 reverse phase; 100 $\times$ 2 mm; 3 μm particle size). Detection was with an LC4C amperometric detector (Bioanalytical Systems, West Lafayette, IN) and a glassy carbon electrode (6 mm diameter) maintained at a potential of +0.6 V. Data for DA and DOPA is expressed in picograms per 20 μl sample. Data for amino acids is presented as a percentage of baseline concentration.

Histological and 5-HT tissue content analyses. All rats were killed by rapid decapitation 1 week after the microdialysis procedure and/or after treatment with MDMA or saline. Brains were removed and rapidly frozen on dry ice. Brains were sectioned in 40 μm increments on a cryostat (-20°C), and probe placements were recorded. Once the probe tract in the striatum was identified, a 400- μm -thick frozen section was taken and tissue within 1 mm of the probe tract was microdissected and stored at -80°C . Tissue in close proximity to the probe tract was taken for analysis to ensure that the dissected tissue was exposed to the drug during reverse dialysis experiments. Striatal DA and 5-HT tissue content was measured by HPLC-EC. Briefly, 300 μl of 0.1 N perchloric acid was added to each sample, the tissue was sonicated and centrifuged at $14,500 \times g$ for 5 min, and the resulting supernatant (20 μl) was injected onto an HPLC system. Protein content was determined using a Bradford assay. DA and 5-HT tissue content were expressed in picograms per microgram of protein.

Temperature measurements. Rectal temperature measurements were taken during all experiments to determine whether any of the local infusion conditions altered MDMA-induced hyperthermia or basal temperatures. During all experiments, ambient temperature was maintained at $22\text{--}24^\circ\text{C}$. Core body temperature was measured hourly using a rectal probe digital thermometer (Thermalert TH-8; Physitemp Instruments, Clifton, NJ).

Statistical analyses. Two-way ANOVAs and ANOVAs with repeated measures were used to compare rats pretreated with MDMA or saline over time and during drug administrations. Student's *t* tests were used to compare saline- and MDMA-injected groups for 5-HT tissue content for animals used in Figures 1 and 8. The experiment depicted in Figure 7A represents an unbalanced design and does not conform to a two-way ANOVA design and analysis. The primary comparison of interest within this design is that between the MDMA/L-tyrosine group and MDMA/L-tyrosine/NSD 1015 group. Therefore, a *t* test was performed to compare these two groups and only these groups within this design.

In all other experiments, Tukey's honestly significant difference tests were used after ANOVAs to determine significant differences between conditions, in which *q* is defined as the Tukey's critical value. Statistical significance was set at $p < 0.05$ for all conditions and experiments.

Results

Tyrosine effects in the striatum

The effect of neurotoxic doses of MDMA (10 mg/kg, i.p., every 2 h for 6 h) on extracellular tyrosine concentrations in striatum was investigated. Systemic treatment of MDMA increased extracellular tyrosine concentrations in the striatum compared with saline-injected controls over time (significant interaction effect, $F_{(9,90)} = 4.426$; $p < 0.05$) (Fig. 1). MDMA acutely increased

Figure 1. The effect of MDMA on extracellular tyrosine concentrations in the striatum. Dialysate samples were collected every hour during 2 h of baseline and 8 h thereafter. Arrows denote injections of MDMA (10 mg/kg per injection, i.p., every 2 h for 4 times, for 6 h) or saline (1 ml/kg for 4 times, i.p., every 2 h for 6 h). MDMA significantly increased tyrosine fivefold from baseline and compared with saline-injected controls ($p < 0.05$). Data are expressed as the mean $\pm$ SEM percentage of baseline values. The basal concentration of tyrosine obtained in dialysate was 5044.36 ± 592.59 pg/20 μ l. $n = 6$ rats per group.

extracellular tyrosine concentrations in striatum fivefold from baseline ($q = 3.807$; $p < 0.05$) and significantly depleted 5-HT by 40% in the striatum 1 week later ($t_{(10)} = 3.827$; $p < 0.05$). 5-HT tissue concentrations were 5.21 ± 0.19 and 3.13 ± 0.41 pg 5-HT/ μ g protein in saline- and MDMA-treated animals, respectively. Although not significant, repeated administrations of saline (1 ml/kg, i.p., every 2 h for 6 h) resulted in a gradual decrease in extracellular tyrosine concentrations from baseline across the 8 h treatment period (Fig. 1). Rats treated with MDMA exhibited consistent hyperthermia throughout the treatment regimen, whereas no effect in temperature was seen in rats treated with saline (data not shown).

Local perfusion of MDMA in striatum

MDMA (100 μ M) was reverse dialyzed into the striatum to determine whether the local administration of MDMA increased extracellular tyrosine concentrations. Local administration of MDMA did not alter extracellular tyrosine concentrations in striatum (Fig. 2) and did not affect 5-HT tissue content 1 week later. 5-HT tissue concentrations were 5.17 ± 0.25 and 5.06 ± 0.40 pg 5-HT/ μ g protein in saline- and MDMA-treated animals, respectively. A steady decline was observed in extracellular tyrosine concentrations in striatum during the infusion with MDMA or Dulbecco's saline (aCSF).

Local perfusion of tyrosine in striatum

To explore the possibility that increased tyrosine concentrations contribute to MDMA-induced 5-HT neurotoxicity, tyrosine was reverse dialyzed into the striatum during systemic administrations of MDMA (10 mg/kg per injection, i.p., every 2 h for 6 h). As in Figure 3A, MDMA decreased 5-HT content compared with saline-injected controls ($q = 8.403$; $p < 0.05$). This decrease was significantly enhanced by the local perfusion of L-tyrosine but not L-valine (significant interaction, $F_{(3,24)} = 30.461$; $p < 0.05$). Specifically, the reverse dialysis of L-tyrosine (500 μ M) exacerbated the MDMA-induced 5-HT depletion in striatum by 30% ($q = 4.328$; $p < 0.05$) (Fig. 3A). L-valine (500 μ M) was infused into the contralateral striatum in each rat as an internal control for the

Figure 2. The effect of local perfusion of MDMA on extracellular tyrosine concentrations. MDMA (100 μ M) was perfused into one striatum and aCSF into the other during dual-probe microdialysis experiments for 2 h prebaseline, 2 h baseline, and 8 h experimental samples. Arrows denote injections of saline (1 ml/kg for 4 times, i.p., every 2 h for 6 h); the inset denotes local infusion conditions. Data are expressed as the mean $\pm$ SEM percentage of baseline values. There are no differences between MDMA and saline-treated rats. $n = 6$ rats per group.

Figure 3. The effect of local perfusion of L-tyrosine or L-valine on striatal tissue concentrations of 5-HT (A) and DA (B) 1 week after systemic treatment of MDMA (10 mg/kg per injection, i.p., every 2 h for 4 times, for 6 h) or saline (1 ml/kg for 4 times, i.p., every 2 h for 6 h). aCSF, L-tyrosine (500 μ M; TYR), or L-valine (500 μ M; VAL) was perfused into one striatum during systemic administration of MDMA for 2 h prebaseline, 2 h baseline, and 8 h experimental samples. Rats were killed 1 week later. * $p < 0.05$ compared with saline (SAL)–aCSF; $\wedge p < 0.05$ compared with all groups (A). $n = 6$ rats per group.

amount of amino acid that was reverse dialyzed. L-valine did not affect MDMA-induced 5-HT depletions. Figure 3B shows DA tissue content for the same rats and conditions. MDMA did not affect DA tissue content after any of the local infusion conditions.

Tyrosine conversion to DOPA

Figure 4 illustrates the production of DOPA measured after incubation of a hydroxyl radical-generating system with various concentrations of L-tyrosine (25, 62.5, 125, and 250 μ M). Increasing tyrosine concentrations resulted in higher concentrations of DOPA over time (significant interaction, $F_{(3,80)} = 23.408$; $p < 0.05$). L-Tyrosine was converted to DOPA in the presence of hydroxyl radicals in a concentration-dependent manner, peaking between 45 and 60 min after incubation of L-tyrosine for all con-

Figure 4. *In vitro* DOPA formation after incubation of an Fe^{2+} /ascorbate hydroxyl radical-generating system based on the Fenton reaction with L-tyrosine in the concentrations of 250, 125, 62.5, and 25 μM . Twenty microliter aliquots were analyzed after reaction times of 5, 15, 30, 45, 60, and 120 min. Data are expressed as picograms per 20 μL of aliquot $\pm$ SEM. * $p < 0.05$ compared with all other concentrations. $n = 5$ –6 reactions per tyrosine concentration.

centrations. Incubation of the reaction mixture with 250 μM L-tyrosine produced the greatest amount of DOPA compared with all other concentrations of tyrosine ($p < 0.05$), whereas the 25 μM concentration produced the least compared with all other concentrations ($p < 0.05$).

Inhibition of aromatic amino acid decarboxylase in striatum

The next set of experiments was performed to address whether the tyrosine-induced enhancement of MDMA-induced 5-HT depletions in striatum was mediated by the conversion of DOPA to DA via AADC. Rats were treated systemically with MDMA or saline as before in the presence or absence of the AADC inhibitor NSD 1015 (100 μM) and L-tyrosine (500 μM). Figure 5A illustrates the extracellular concentrations of DA measured before and during administration of MDMA. There was a differential effect of the local perfusion condition to increase extracellular DA over time ($F_{(21,206)} = 3.518$; $p < 0.05$). Tyrosine potentiated the MDMA-induced increases in extracellular DA compared with aCSF controls ($q = 5.569$; $p < 0.05$). NSD 1015 completely blocked the MDMA-induced increase in DA ($q = 5.910$; $p < 0.05$) and prevented the tyrosine-induced enhancement of MDMA-induced increases in extracellular DA in striatum ($q = 9.459$; $p < 0.05$) (Fig. 5A).

Figure 5B depicts average striatal DA concentrations from saline-injected rats with the same infusion conditions as those in Figure 5A. NSD 1015 significantly decreased basal DA in the presence or absence of L-tyrosine ($F_{(3,20)} = 7.483$; $p < 0.05$). Specifically, NSD 1015 decreased basal DA in striatum compared with aCSF ($q = 4.044$; $p < 0.05$) and L-tyrosine ($q = 6.388$; $p < 0.05$). The coinfusion of L-tyrosine with NSD 1015 partially reversed the decrease in extracellular DA concentrations produced by NSD 1015 such that the concentrations were not different from the infusion of aCSF alone. The infusion of L-tyrosine did not significantly change basal DA concentrations in striatum when compared with the infusion of aCSF.

Systemic administration of MDMA and striatal 5-HT content

The effect of the local perfusion of NSD 1015, L-tyrosine, or the combination of NSD 1015 and L-tyrosine on 5-HT tissue content in striatum 1 week after the systemic administration of MDMA or

Figure 5. Acute extracellular DA concentrations were assessed in striata of rats treated with MDMA (10 mg/kg per injection for 4 times, i.p., every 2 h for 6 h) (A) or saline (1 ml/kg for 4 times, i.p., every 2 h for 6 h) (B), and 5-HT (C) and DA (D) tissue content was measured 1 week later. All probes were reverse dialyzed with aCSF, NSD 1015 (100 μM), L-tyrosine (500 μM ; TYR), or the combination of L-tyrosine and NSD 1015 (TYR + NSD). A, NSD 1015 attenuated the increase in extracellular DA concentrations after MDMA and the L-tyrosine-induced enhancement of extracellular DA after MDMA. Arrows denote injections of MDMA. The inset denotes infusion conditions. Data are expressed in picograms of DA per 20 μL of dialysate $\pm$ SEM. TYR infusion group is significantly greater than the NSD 1015 and TYR + NSD 1015 groups at all time points and different from aCSF during hours 1–5 ($p < 0.05$); NSD 1015 infusion is significantly less than TYR and aCSF groups at all time points and less than TYR + NSD 1015 during hours 1–5 ($p < 0.05$). B, Local perfusion of NSD 1015 decreased basal DA. Data are expressed as average of the basal DA concentration across two baseline samples in picograms per 20 μL of dialysate $\pm$ SEM. * $p < 0.05$ compared with aCSF infusion and L-tyrosine; $\wedge p < 0.05$ compared with L-tyrosine infusion. C, Reverse dialysis of NSD 1015 blocked MDMA-induced 5-HT depletions in striatum as well as the L-tyrosine-induced enhancement of these depletions. Inset denotes systemic injection conditions. D, None of the infusion conditions affected DA tissue content after MDMA. The inset denotes systemic injection conditions. Data in C and D are expressed in 5-HT or DA picograms per microgram of protein $\pm$ SEM, respectively. * $p < 0.05$ compared with saline-treated control animals; $\wedge p < 0.05$ compared with all other infusion groups in MDMA-treated animals. $n = 6$ –8 rats per group.

saline was also examined. As illustrated in Figure 5C, MDMA significantly decreased 5-HT tissue content compared with saline-injected controls ($F_{(1,55)} = 51.399$; $p < 0.05$). This long-term decrease in 5-HT tissue content was differentially affected by NSD 1015, L-tyrosine, or the combination of NSD 1015 and L-tyrosine (significant interaction, $F_{(3,55)} = 4.637$; $p < 0.05$). Specifically, MDMA (10 mg/kg per injection, i.p., every 2 h for 8 h) decreased striatal 5-HT tissue content 1 week later compared with saline-injected controls in the aCSF infusion condition ($q = 4.003$; $p < 0.05$). Reverse dialysis of NSD 1015 blocked ($q = 3.791$; $p < 0.05$), whereas L-tyrosine exacerbated ($q = 4.200$; $p < 0.05$), the MDMA-induced decrease in striatal 5-HT tissue content (Fig. 5C). The coinfusion of NSD 1015 with L-tyrosine blocked the exacerbation of MDMA-induced 5-HT depletions induced by L-tyrosine alone ($q = 3.773$; $p < 0.05$). None of these infusion conditions or the systemic administration of MDMA affected DA tissue content in striatum (Fig. 5D).

Figure 6. The effect of local administration of MDMA alone or in conjunction with L-tyrosine (TYR) or L-valine (VAL) on tissue concentrations of 5-HT and DA 1 week later. The inset denotes infusion conditions for both **A** and **B**. aCSF or MDMA (100 μ M) was perfused alone or in combination with L-tyrosine (500 μ M) or L-valine (500 μ M) for 12 h, and all rats were treated with saline (1 ml/kg for 4 times, i.p., every 2 h for 6 h). Tissue concentrations of 5-HT (**A**) and DA (**B**) were determined in the immediate vicinity of the probe tract 1 week later. Data are expressed in 5-HT or DA picograms per microgram of protein $\pm$ SEM, **A** and **B**, respectively. * $p < 0.05$ compared with MDMA and aCSF groups. $n = 6$ rats per group.

Local perfusion of MDMA and 5-HT content

To determine whether the coinfusion of L-tyrosine and MDMA could result in long-term depletions of striatal 5-HT, MDMA (100 μ M) was reverse dialyzed alone or in combination with L-tyrosine (500 μ M) or L-valine (500 μ M) into the striatum. The decrease in 5-HT content was dependent on whether MDMA or L-tyrosine was infused (significant interaction, $F_{(3,16)} = 5.578$; $p < 0.05$). Although MDMA alone or in combination with L-valine did not affect 5-HT tissue content in striatum 1 week later, coinfusion of MDMA and L-tyrosine produced a significant decrease in 5-HT tissue content in striatum ($q = 5.472$; $p < 0.05$) (Fig. 6A). None of the infusion conditions affected DA tissue content of the striatum 1 week later (Fig. 6B).

To assess whether the long-term depletion of 5-HT observed after the coinfusion of MDMA and L-tyrosine was dependent on AADC, NSD 1015 (100 μ M) was coinfused with MDMA (100 μ M) and L-tyrosine (500 μ M) (Fig. 7). Consistent with Figure 6, MDMA alone did not change striatal 5-HT content. In contrast, the effect of the infusion of MDMA on 5-HT content was dependent on the presence of L-tyrosine ($F_{(1,29)} = 6.221$; $p < 0.05$) (Fig. 7A). The combined infusion of MDMA and L-tyrosine significantly decreased 5-HT tissue content compared with the infusion of MDMA alone ($q = 4.360$; $p < 0.05$) and L-tyrosine alone ($q = 3.985$; $p < 0.05$). The combination of NSD 1015 with MDMA and L-tyrosine blocked the depletion of 5-HT produced by the MDMA and L-tyrosine combination ($t_{(14)} = 4.032$; $p < 0.05$). None of the infusion conditions changed DA tissue content (Fig. 7B). The local infusion of MDMA or any of the infusion conditions used above did not produce changes in core body temperature (data not shown).

Tyrosine effects in the hippocampus

To examine the influence of L-tyrosine on MDMA-induced 5-HT depletions in a region sparsely innervated by DA, similar experiments were performed in the ventral hippocampus. MDMA increased extracellular tyrosine concentrations in the hippocampus compared with saline-injected control rats over time (significant interaction, $F_{(9,82)} = 7.959$; $p < 0.05$). MDMA (10 mg/kg per injection, i.p., every 2 h for 6 h) increased extracellular tyrosine concentrations 2.5-fold above saline-injected control rats ($q =$

Figure 7. The effect of local perfusion of MDMA alone or in combination with L-tyrosine (TYR) and/or NSD 1015 on the tissue concentrations of 5-HT and DA in the striatum 1 week later. All rats were treated with saline (1 ml/kg for 4 times, i.p., every 2 h for 6 h). aCSF or MDMA (100 μ M) was perfused alone or in combination with L-tyrosine (500 μ M) and/or NSD 1015 (100 μ M) for 12 h, and tissue concentrations of 5-HT (**A**) and DA (**B**) were determined in the immediate vicinity of the probe tract 1 week later. The inset denotes local perfusion conditions for both **A** and **B**. Data are expressed as picograms per microgram of protein $\pm$ SEM. * $p < 0.05$ compared with all groups. $n = 6$ rats per group.

Figure 8. The effect of neurotoxic doses of MDMA on extracellular tyrosine concentrations in ventral hippocampus examined 1 week after the dialysis experiment. Rats were treated with neurotoxic doses of MDMA (10 mg/kg for 4 times, i.p., every 2 h for 6 h) or saline (1 ml/kg for 4 times, i.p., every 2 h for 6 h). **A**, Hourly hippocampal tyrosine concentrations after MDMA or saline. Arrows denote injections of MDMA or saline. Data are expressed as a percentage of the basal tyrosine concentration measured in dialysate (picograms per 20 μ l of aliquot) $\pm$ SEM. MDMA significantly increased extracellular tyrosine concentrations compared with saline-injected control rats ($p < 0.05$). The basal concentration of tyrosine obtained in dialysate was 4627.84 ± 484.73 pg/20 μ l. $n = 6$ rats per group.

8.756; $p < 0.05$) (Fig. 8). Similar to that seen in striatum, injections of saline (1 ml/kg, i.p., every 2 h for 6 h) resulted in a gradual, but nonsignificant, decrease in extracellular tyrosine concentrations from baseline across the 8 h treatment period (Fig. 8A). This dosing regimen of MDMA significantly depleted 5-HT content in the hippocampus by 60% 1 week later ($t_{(11)} = 5.885$; $p < 0.05$). 5-HT tissue concentrations were 5.11 ± 0.40 and 2.37 ± 0.25 pg 5-HT/ μ g protein in saline- and MDMA-treated animals, respectively.

The role of tyrosine-induced changes in extracellular DA in

Figure 9. Effect of systemic administration of MDMA on acute extracellular DA concentrations and long-term 5-HT tissue concentrations in hippocampus. Rats were treated with MDMA (10 mg/kg per injection, i.p., every 2 h for 4 times, for 6 h), and extracellular DA concentrations were determined hourly in dialysate. The inset denotes infusion conditions (**A**). **A**, Reverse dialysis of L-tyrosine (TYR) potentiates and NSD 1015 prevents the increase in extracellular DA concentrations after MDMA. Coinfusion of L-tyrosine and NSD 1015 or an infusion of α MPT (AMPT) produce extracellular DA concentrations similar to aCSF. Arrows denote injections of MDMA. * $p < 0.05$ compared with all other groups. **B**, Reverse dialysis of the AADC inhibitor NSD 1015 (NSD) blocked MDMA-induced 5-HT striatal depletions (10 mg/kg, i.p., every 2 h for 4 times, over 6 h), as well as the tyrosine-induced enhancement of these depletions. Reverse dialysis of α MPT produces a 5-HT depletion similar in degree to aCSF. The inset denotes systemic treatment (**B**). * $p < 0.05$ compared with saline control; $\wedge p < 0.05$ compared with all other infusion conditions in MDMA-treated animals except α MPT.

mediating long-term MDMA-induced 5-HT depletions in hippocampus was investigated. Figure 9A shows that repeated injections of MDMA acutely increased extracellular DA concentrations in the hippocampus ($F_{(4,135)} = 9.618$; $p < 0.05$). The reverse dialysis of L-tyrosine (500 μ M) significantly potentiated the MDMA-induced increase in DA ($q = 4.690$; $p < 0.05$). NSD 1015 (100 μ M) prevented this MDMA-induced increase such that DA concentrations were significantly different between the aCSF and NSD 1015 perfusion conditions ($q = 5.310$; $p < 0.05$). In addition, there was a significant difference between the potentiation of the MDMA-induced DA release by the infusion of L-tyrosine alone and the combination of L-tyrosine and NSD 1015 ($q = 4.688$; $p < 0.05$). Reverse dialysis of the tyrosine hydroxylase inhibitor α MPT did not alter the MDMA-induced increases in extracellular DA concentrations (Fig. 9A).

Figure 9B depicts the corresponding long-term changes in 5-HT content in the hippocampus 1 week after systemic administration of MDMA. Systemic MDMA treatment decreased 5-HT

tissue content compared with saline-injected controls ($F_{(1,46)} = 127.356$; $p < 0.05$). This effect was differentially dependent on the local infusion of L-tyrosine and/or NSD 1015 ($F_{(4,46)} = 5.589$; $p < 0.05$). The reverse dialysis of NSD 1015 blocked or attenuated the MDMA-induced 5-HT depletions in aCSF controls ($q = 6.014$; $p < 0.05$) and the L-tyrosine-infused group ($q = 4.274$; $p < 0.05$), respectively. The reverse dialysis of α MPT did not affect the MDMA-induced 5-HT depletions in the ventral hippocampus.

Discussion

The contribution of L-tyrosine to MDMA-induced 5-HT depletions in the striatum and hippocampus was investigated. The systemic administration of MDMA increased the extracellular concentration of tyrosine in the brain that, in turn, contributed to the selective long-term depletions of 5-HT in an AADC-dependent manner.

The increase in tyrosine after the systemic administration of MDMA is not attributable to direct effects of MDMA in the brain because the reverse dialysis of MDMA into the striatum did not change extracellular tyrosine concentrations (Fig. 2). Several possibilities may explain the increase in brain tyrosine after the systemic administration of MDMA. MDMA-induced norepinephrine (NE) release (Lavelle et al., 1999), stimulation of peripheral β -adrenergic receptors, and the subsequent increase in LNAA transport into the brain (Eriksson and Carlsson, 1988; Takao et al., 1992) could increase brain tyrosine. Second, stimulation of adrenergic receptors by noradrenaline may facilitate the breakdown of skeletal muscle and rhabdomyolysis (Sprague et al., 2004) to increase circulating amino acids (e.g., tyrosine) and, subsequently, their concentrations in brain. Finally, MDMA may disrupt the blood–brain barrier (Bankson et al., 2005), such that the normally regulated transport of tyrosine from the brain to the periphery is impaired.

The underlying mechanism of the differential increase in tyrosine between the striatum and hippocampus after MDMA is unknown (Figs. 1, 8) but may be related to the differential density of DA innervation of these regions (Versteeg et al., 1976). Another possibility may be a heterogeneity of active transport systems for tyrosine by the blood–brain barrier endothelial cells within the striatum and hippocampus. Somewhat inconsistent with the current findings is that greater increases in tyrosine within the hippocampus compared with the striatum were observed after systemic tyrosine administration (Morre et al., 1980). Clearly, the increases in brain tyrosine produced by MDMA are mediated through different mechanisms other than simply increasing the systemic circulating concentrations of tyrosine alone. Differences in regional cerebral blood flow between the striatum and hippocampus (Kelly et al., 1994) that influence the availability of tyrosine for transport across the blood–brain barrier may also account for the regional differences in the increase in tyrosine after MDMA. Moreover, the systemic administration of MDMA appears critical for mediating the increases in brain tyrosine because only the systemic and not the local administration of MDMA increased brain extracellular tyrosine concentrations (Fig. 2A).

Elevations in brain tyrosine appear to contribute to the long-term 5-HT depletions in the striatum and hippocampus after MDMA. The reverse dialysis of L-tyrosine exacerbated 5-HT depletions after the systemic administration of MDMA and depleted striatal 5-HT with the local perfusion of MDMA. It should be noted that the choice of the concentration of tyrosine (500 μ M) is an estimate based on an extrapolation from known basal concentrations of tyrosine in the brain, the magnitude of increase

produced by MDMA, and the semipermeability of the dialysis membrane to tyrosine. Regardless, the concentration of tyrosine alone had no effect on the content of 5-HT or DA but only depleted 5-HT when perfused with the local administration of MDMA. MDMA by itself did not have any effect on tyrosine (Fig. 2) and did not produce a long-term depletion of 5-HT, despite markedly elevated extracellular DA concentrations as observed previously (Esteban et al., 2001; Nixdorf et al., 2001). Thus, locally applied MDMA is only toxic to 5-HT in the presence of high tyrosine concentrations (Figs. 6A, 7A) such as those observed after systemic MDMA. Furthermore, the enhancement of MDMA-induced toxicity by L-tyrosine is not attributable to a general increase in LNAA concentrations because the reverse dialysis of another LNAA, L-valine, with MDMA did not change 5-HT content (Figs. 3A, 6A). Collectively, these data suggest that high concentrations of tyrosine that are approached after drug administration can be neurotoxic in the presence of MDMA.

The ability of L-tyrosine to enhance MDMA-induced increases in extracellular DA further supports the role of DA in mediating the damage to 5-HT neurons (Figs. 5A, 9A). Although striatal DA synthesis generally does not change with increases in tyrosine (Carlsson et al., 1972; Bongiovanni et al., 2003) (Fig. 5B), the enhancement of MDMA-induced DA by tyrosine is consistent with reports that tyrosine can increase DA synthesis when DA neuron activity is enhanced (Scally et al., 1977; Sved and Fernstrom, 1981; Daring et al., 1989).

Tyrosine-derived DA synthesis may not be mediated through the activation of TH. The reverse dialysis of the competitive TH inhibitor α MPT did not affect MDMA-induced increases in extracellular DA, MDMA-induced hyperthermia, or the long-term depletions of 5-HT in hippocampus (Fig. 9), consistent with a previous demonstration that α MPT did not protect against MDMA-induced 5-HT deficits when α MPT-induced hypothermia was prevented (Yuan et al., 2002). The concentration of α MPT (100 μ M) was likely sufficient to inhibit TH despite the increased tyrosine concentrations because it is fivefold higher than the K_m of tyrosine for TH (Bakhit et al., 1980) and was perfused for 3.5 h before the increases in tyrosine occur.

An alternate explanation for the hydroxylation of tyrosine that does not require TH is that increases in tyrosine during pro-oxidant conditions produced by MDMA (Colado et al., 1997; Shankaran et al., 1999) may nonenzymatically convert tyrosine to DOPA. Indeed, tyrosine is converted to DOPA in the presence of hydroxyl radicals independent of TH or other enzymes (Fig. 4). Consequently, MDMA-induced increases in extracellular DA do not change after the local inhibition of TH (Fig. 9A). Thus, tyrosine taken up by nerve terminals (Morre and Wurtman, 1981) can be converted to DOPA in the presence of hydroxyl free radicals.

The hypothesis of increased hydroxylation of tyrosine by hydroxyl radicals to DOPA assumes the preexistence of hydroxyl radicals. Activation of the serotonin transporter can contribute to hydroxyl radical formation after MDMA (Shankaran et al., 1999). In addition, hyperthermia, such as that produced by MDMA (Nash et al., 1988; Shankaran et al., 2001), can produce hydroxyl radicals (Globus et al., 1995), and MDMA itself can be metabolized to form quinones (Hiramatsu et al., 1990), toxic metabolites (Jones et al., 2005), and further promote free radical production (Graham et al., 1978). Thus, preexistent increases in hydroxyl radicals produced by MDMA from multiple sources alone may not be sufficient to damage 5-HT terminals but are necessary to promote a tyrosine-dependent oxidant stress to 5-HT terminals.

DOPA formed from tyrosine is most likely converted to DA by AADC endogenous to 5-HT terminals. MDMA-induced increases in extracellular DA and its augmentation by tyrosine are blocked by the inhibition of AADC (Fig. 5A). Moreover, the prevention of the MDMA and tyrosine-induced increases in DA by AADC inhibition also attenuate the long-term decreases in 5-HT content after MDMA alone and after MDMA with tyrosine (Figs. 5C, 9B). Thus, AADC appears critical for MDMA-induced 5-HT depletions.

Although the accumulation of DOPA after NSD 1015 can lead to cytotoxic DOPA quinones that, in turn, damage 5-HT terminals (Graham, 1978), the metabolism of DA by MAO-B appears important. Inhibition of MAO-B by L-deprenyl (Sprague and Nichols, 1995) or gene knockout (Falk et al., 2002) protects against MDMA-induced damage to 5-HT terminals. Thus, the metabolism of DA promotes oxidative stress (Cohen, 1987) within 5-HT terminals and could deplete antioxidant stores (Shankaran et al., 2001) to produce damage.

The difference between the magnitude of tyrosine increase and 5-HT depletion observed may be related to the differential presence of DA endogenous to each region (Versteeg et al., 1976). The enhancement of MDMA-induced increases in DA by L-tyrosine (Figs. 5A, 9A) suggests that tyrosine is taken up into DA terminals for DA synthesis. The magnitude of tyrosine increase in striatum by MDMA may overcome the biosynthetic capacity of DA terminals and overflow to promote tyrosine transport into 5-HT terminals for the nonenzymatic hydroxylation of tyrosine to DOPA and conversion to DA to mediate 5-HT terminal damage. The absence of DA terminals in the hippocampus may permit the smaller but significant increases in tyrosine to have a greater neurotoxic impact because of the lower capacity of the hippocampus to enzymatically metabolize tyrosine to DA for neurotransmission and subsequent enzymatic degradation. Thus, DA terminals of the striatum may buffer much of the increased tyrosine after MDMA, whereas the hippocampus may be more vulnerable to DA-derived pro-oxidant species because of the paucity of DA terminals inherent to this region.

The partial attenuation of MDMA-induced toxicity by NSD 1015 (Figs. 5, 9) may also be explained by the fact that tyrosine may undergo fates other than the conversion to DOPA and DA. One possibility is that tyrosine is converted by AADC to tyramine (Dyck et al., 1983; Juorio and Yu, 1985). Tyramine can then be metabolized by MAO-B (Schoepp and Azzaro, 1981) to oxidatively damage mitochondria (Hauptmann et al., 1996). Another possibility is that tyrosine itself can be oxidized by ferryl ions to produce tyrosyl radicals (Metodiowa and Dunford, 1993). These tyrosine-derived radical species can interact with transition metals to promote lipid peroxidation (Savenkova et al., 1994) and damage to DNA and proteins (Pichorner et al., 1995).

The selectivity of tyrosine toxicity to 5-HT versus DA or NE terminals may be explained by tyrosine being the natural precursor for transmitter synthesis in DA and NE but not 5-HT terminals. Therefore, 5-HT terminals may have less capacity to metabolize abnormally high concentrations of tyrosine or DA to benign metabolites. Thus, tyrosyl radicals, tyrosine-derived DOPA quinones, or MAO-B derived hydroxyl radicals may be more easily formed and accumulate in 5-HT terminals during high concentrations of tyrosine. Additionally, the known fourfold greater selectivity of MDMA to increase 5-HT compared with DA release (Setola et al., 2003) may partly explain the selectivity of MDMA for 5-HT terminals.

In summary, MDMA increases the concentration of tyrosine in the brain that contributes to long-term depletions of 5-HT.

The ability of tyrosine to mediate MDMA-induced 5-HT depletions appears to occur through the nonenzymatic hydroxylation of tyrosine to DOPA and then to DA via AADC within 5-HT neurons. The present findings highlight the importance of tyrosine and support a role for DA in mediating toxicity to 5-HT terminals after MDMA while providing a novel, alternate mechanism for how DA can mediate long-term 5-HT depletions in regions with sparse catecholamine innervation.

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