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DEPRESSION OF RAT BRAIN TRYPTOPHAN HYDROXYLASE ACTIVITY FOLLOWING THE ACUTE ADMINISTRATION OF METHYLENEDIOXYMETHAMPHETAMINE

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Abstract—The psychotomimetic agent, methylenedioxymethamphetamine, produced a rapid, persistent and dose-dependent reduction in cortical tryptophan hydroxylase activity when administered acutely to rats. This effect did not occur *in vitro* and did not require *N*-demethylase activity in the whole animal. Kinetic analysis revealed the loss of enzyme activity to be due to an alteration in V_{max} with no change in the affinity of the enzyme for either its cofactor or substrate. Coadministration of the serotonin (5-HT) uptake inhibitor, citalopram, only partially antagonized the loss of tryptophan hydroxylase activity 3 hr after methylenedioxymethamphetamine, but completely prevented the loss of cortical 5-HT. Recovery of enzyme activity did occur by 1 week if the neurotoxic effect of methylenedioxymethamphetamine was blocked by fluoxetine. The effect of methylenedioxymethamphetamine on 5-HT synthesis was not affected by pretreatment with alpha-methyl-*p*-tyrosine, reserpine or yohimbine. Ketanserine and methiothepin, 5-HT receptor antagonists, did partially block the methylenedioxymethamphetamine-induced loss of tryptophan hydroxylase activity, suggesting a possible role for neurotransmitter release in the acute effects of the drug on enzyme activity.

The amphetamine analogue, methylenedioxymethamphetamine (MDMA), produces effects in the rat brain reminiscent of the serotonergic neurotoxic *p*-chloroamphetamine (PCA). Acute administration of MDMA causes both an immediate and a long-term decrease in brain serotonin (5-HT) concentrations which is still present 1 week after the drug [1–3]. The initial effects of MDMA on 5-HT concentrations appear to be reversible and have been suggested to be due to an inhibition of transmitter synthesis coupled with an increase in carrier-mediated efflux of 5-HT [3]. Unlike the long-term effects of MDMA, these immediate effects of the drug do not have a stereochemical requirement in that either the (+)- or (−)-enantiomer will produce similar depletions of 5-HT [2, 3]. In contrast, the effect of MDMA measured 1 week following drug administration is a property of the (+)-stereoisomer of the drug and has been associated with a decrease in the number of [^{3}H]5-HT uptake sites, suggesting nerve terminal damage [3]. All of these characteristics are very similar to those described for the effects of PCA on serotonergic neurons in the rat brain [4–6].

Although the basis of the long-term depression of 5-HT concentrations seems to be related to the development of a form of neurotoxicity, the neurochemical basis for the immediate loss of brain 5-HT is less clear. Stone *et al.* [7] have demonstrated recently that activity of the rate-limiting enzyme in 5-HT synthesis, tryptophan hydroxylase (TPH), is reduced significantly following either acute or subacute treatment with MDMA. However, the mechanism by which MDMA inhibits TPH activity remains to be elucidated. These studies were undertaken to further examine the effects of MDMA on 5-HT synthesis and, in particular, to focus on the mechanism of the initial effects of the drug on serotonergic neurons.

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METHODS

Drug administration. Male Sprague–Dawley rats (200–250 g) were maintained on a 12-hr light–dark cycle and allowed free access to food and water. All drugs were administered as the free base in saline by subcutaneous injection unless stated otherwise. Reserpine and piperonyl butoxide were dissolved in 1% acetic acid and corn oil, respectively. Animals were killed by decapitation, and their brain was dissected on ice. All tissues were stored at −80° until assayed.

MDMA was synthesized according to Braun *et al.* [8] as described previously [1–3]. Other drugs used and their sources were: tryptophan (Trp), reserpine, yohimbine, and alpha-methyl-*p*-tyrosine (Sigma Chemical Co., St. Louis, MO), fluoxetine (Lilly Research Laboratories, Indianapolis, IN), citalopram (H. Lundbeck & Co., Copenhagen, Netherlands), piperonyl butoxide (Fluka Chemical Co., Ronkonkoma, NY), methiothepin (Hoffmann–La Roche, Inc., Nutley, NJ), and ketanserine (Research Biochemicals, Inc., Wayland, MA).

Cortical indole concentrations. Samples of cerebral cortex corresponding to parietal and frontal cortex

were dissected and immediately frozen on dry ice. Tissues were stored at −80° until assayed. Cortical 5-HT concentrations were determined using high performance liquid chromatography with electrochemical detection. After being weighed, the samples were homogenized in 1 ml of mobile phase containing monochloroacetic acid (0.15 M), EDTA (0.2 mM), heptyl sulfonic acid (1 g/l) and 10% methanol at pH 2.9. *N*-Acetylserotonin (0.1 μg/ml) was added as an internal standard to allow recoveries to be determined. After centrifugation (30,000 *g*, 15 min), 25-μl aliquots of the supernatant fractions were injected directly onto a Waters 5 μm Nov-a-pak C-18 column (Milford, MA). Detection was by means of an ESA Coulochem model 5100A detector (Bedford, MA) using a potential of +0.40 V and a guard potential of +0.05 V. 5-HT was quantitated by comparison with standards of known concentration using a Spectra Physics SP 4270 integrator (San Jose, CA).

TPH activity. Cortical TPH activity was determined in a modified $^{14}CO_2$ trapping assay as described by Hotchkiss *et al.* [9]. Briefly, samples of cerebral cortex were weighed and homogenized in 0.5 ml of cold 50 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), 0.2% Triton X-100 and 6 mM dithiothreitol at pH 7.4. After centrifugation at 27,000 *g* for 15 min, duplicate 7.5-μl aliquots of each supernatant fraction were added to 5 μl of reaction mixture containing: Hepes, pH 7.4 (9.6 mM), pyridoxal phosphate (0.04 mM), DL-6-methyltetrahydropterine (6-MPH_4, 1.4 mM), [1-^{14}C]tryptophan (Trp, 0.12 mM, sp. act. approximately 6 mCi/mmol) and excess l-aromatic amino acid decarboxylase partially purified from guinea pig kidney as described by Lovenberg and Engelman [10]. After a 30-min incubation at 37° in sealed tubes, the reaction was stopped on ice, and dissolved $^{14}CO_2$ was liberated by injection of 0.1 ml of 5 N H_2SO_4. Small strips of filter paper (2 × 0.5 cm) spotted with 50 μl of hyamine hydroxide were used as CO_2 traps. After a 90-min incubation at 37° to completely trap all evolved $^{14}CO_2$, the filter papers were transferred to vials containing Econofluor-2 scintillation cocktail (New England Nuclear, Boston, MA). Samples were counted on a Beckman LS 5801 counter with quench correction. After correction for blanks (minus 6-MPH_4), specific activity and tissue weight, the data were expressed as nmol Trp oxidized per g per hr. All reagents were from the Sigma Chemical Co. [1-^{14}C]Trp was purchased from New England Nuclear.

In experiments determining the kinetic parameters of TPH, the concentration of either 6-MPH_4 or Trp was held constant while the other was varied. A saturating 6-MPH_4 concentration of 1.36 mM was selected based upon the 0.120 mM K_m reported by Weekley *et al.* [11]. This concentration was six times the K_m determined for our crude enzyme preparation. However, technical considerations in the $^{14}CO_2$-trapping assay precluded using saturating Trp concentrations. The K_m of TPH for Trp determined using cortical homogenates was approximately 0.3 mM. Examination of the double-reciprocal plot for Trp (see Fig. 3A) indicated that 0.6 mM Trp would yield a V_{max} of 86 units when 6-MPH_4 concentrations were varied. The V_{max} of TPH actually determined for 6-MPH_4 at 0.6 mM Trp was 70 ± 9 units (see Fig. 3B) which is in reasonable agreement with the theoretical value.

RESULTS

Effect of MDMA on cortical TPH activity: Acute dose response and time course. Animals were administered MDMA at various doses and killed 3 hr later for the determination of cortical TPH activity. As shown in Fig. 1A, MDMA produced a dramatic and dose-dependent decrease in the activity of the cortical enzyme. At a dose of 5 mg/kg, enzyme activity was reduced to 42% of control values. Higher doses of 10, 15 or 20 mg/kg all reduced cortical TPH activity to approximately 22% of control, indicating a plateau for this effect of the drug.

The 20 mg/kg dose of MDMA was used to study the time course of the change in TPH activity following MDMA administration. As shown in Fig. 1B, cortical TPH activity rapidly decreased after drug administration with the decline in activity gradually leveling out by 12 hr near 20% of control. There was little or no recovery of enzyme activity by 24 hr; however, 7 days after the MDMA, TPH activity had increased to 72% of control. Although increased from 3 hr, this value still represented a significant depression in enzyme activity compared to controls.

Direct effect of MDMA on TPH activity in vitro. The high doses of drug required to produce the

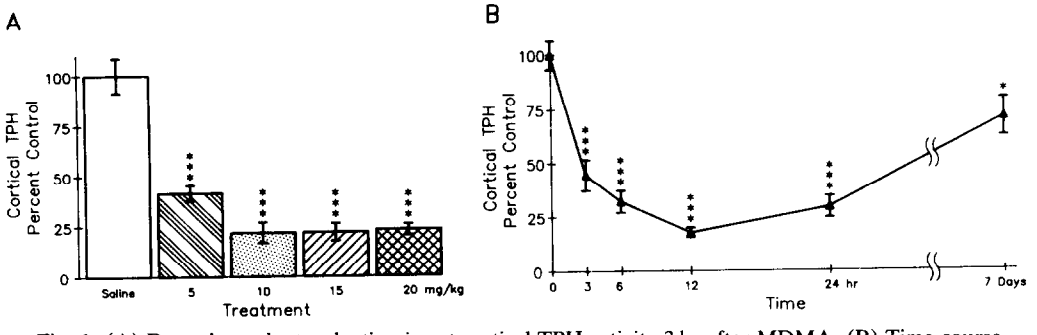

Fig. 1. (A) Dose-dependent reduction in rat cortical TPH activity 3 hr after MDMA. (B) Time course showing the loss of cortical TPH activity after MDMA administration (20 mg/kg). Absolute enzyme activity in saline controls was 31.9 ± 6.0 nmol oxidized/g tissue/hr. Values are the mean ± SEM for five animals. Key: (*) $P < 0.05$ and (***) $P < 0.001$ versus saline control by the two-tailed Student's *t*-test.

Table 1. Effect of MDMA on TPH activity *in vitro*

[MDMA] in assay (μM)	Cortical TPH activity (nmol/g tissue/hr)	% Control
0 (control)	23.1 ± 2.6	100 ± 11.3
1	29.0 ± 0.7	125.4 ± 3.0
10	23.7 ± 3.6	102.6 ± 15.4
100	23.7 ± 1.5	102.5 ± 6.3

MDMA was added directly to the enzyme preparation to give the indicated concentrations. TPH activity was then determined as described in Methods. Each value is the mean ± SEM for three determinations.

measured effects of MDMA on TPH activity (10–20 mg/kg) suggested that a direct effect of the drug on enzyme activity was possible. To examine this question, MDMA was added directly to cortical homogenates from control animals at concentrations similar to those expected *in vivo*. Assuming a whole body distribution of MDMA, a maximum brain concentration of approximately 90 μM would be achieved at a dose of 20 mg/kg. The direct addition of MDMA to brain homogenates at concentrations up to 100 μM had no effect on TPH activity, as shown in Table 1.

Effect of piperonyl butoxide on MDMA-induced changes in serotonin synthesis. The *N*-desmethyl analog of MDMA produces a depression of 5-HT synthesis identical to that observed after acute administration of MDMA [7, 12]. To determine if the *in vivo* conversion of MDMA to methylenedioxyamphetamine (MDA) could play a role in the neurochemical response of serotonergic neurons to MDMA, the potential metabolism of MDMA to MDA was blocked using a well known inhibitor of *N*-demethylase activity, piperonyl butoxide [13]. In two experiments, rats were pretreated 1 or 4 hr prior to MDMA with piperonyl butoxide (1000 mg/kg, i.p.). As shown in Fig. 2, piperonyl butoxide alone had no effect on TPH activity or 5-HT concentrations. MDMA (10 mg/kg) alone reduced enzyme activity and 5-HT concentrations to 35 and 18% of control respectively. When animals were pretreated with piperonyl butoxide 1 hr prior to MDMA, cortical TPH activity and 5-HT concentrations were still reduced to 57 and 25% of control respectively. Earlier pretreatment with piperonyl butoxide yielded similar results (not shown).

Kinetic analysis of the change in cortical TPH activity. To determine if the decrease in TPH activity immediately following MDMA treatment was due simply to a change in the affinity for one of its substrates, the K_m and maximum velocity (V_{max}) of the enzyme from control and drug-treated animals were determined for both Trp and the hydroxylase cofactor, 6-MPH_4. Cortical tissue samples from five saline or MDMA (10 mg/kg) treated rats killed 3 hr after drug administration were used for these experiments. 6-MPH_4 was held constant at 1.36×10^{-3} M, and the Trp concentration was varied between 0.04×10^{-3} and 0.4×10^{-3} M to produce the double-reciprocal plot shown in Fig. 3A. MDMA treatment decreased the V_{max} of TPH by 50% with no change in the affinity for Trp. A similar change was observed for the effect of MDMA on TPH activity with respect to 6-MPH_4 (Fig. 3B). Again maximum enzyme activity was reduced by approximately 50% with no significant change in the affinity of the enzyme for its cofactor. Due to the weight given low substrate concentrations by the double-reciprocal method, the results shown in Fig. 3 were also analyzed by an Eadie–Hofstee plot (V versus $V/[S]$) which yielded identical results.

Effect of 5-HT uptake inhibitors on the loss of TPH activity following MDMA. Figure 4A shows that coadministration of the 5-HT uptake inhibitor, citalopram (2 mg/kg), with MDMA (10 mg/kg) significantly antagonized the decrease in TPH activity measured 3 hr following MDMA administration. MDMA alone reduced enzyme activity to approximately 22% of control. The addition of citalopram

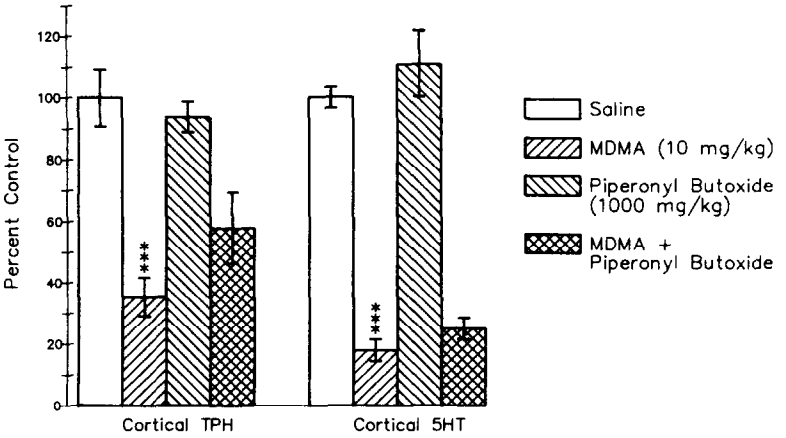

Fig. 2. Effect of the *N*-demethylase inhibition by piperonyl butoxide on MDMA-induced reduction in cortical TPH activity and 5-HT concentrations at 3 hr. Piperonyl butoxide (1000 mg/kg) was administered 1 hr prior to MDMA. Absolute values for control enzyme activity and 5-HT concentrations were 41.0 ± 3.8 nmol oxidized/g tissue/hr and 0.28 ± 0.01 μg/g tissue respectively. Values are the mean ± SEM for five animals. Key: (***) $P < 0.001$ versus saline.

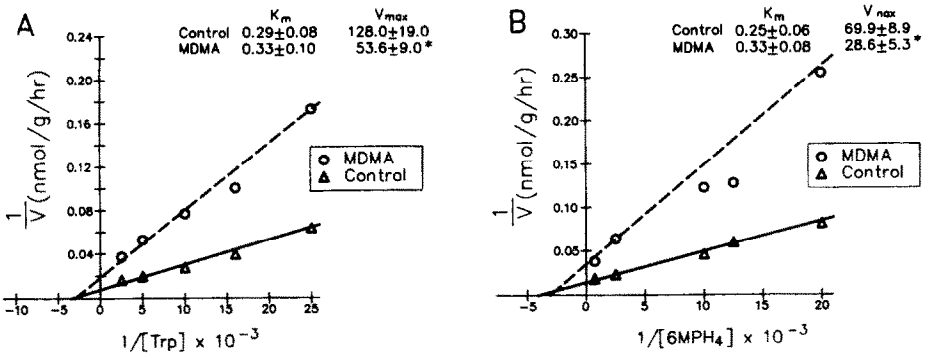

Fig. 3. Lineweaver–Burk plots illustrating the MDMA-induced changes in the kinetic parameters of cortical TPH with respect to Trp (A) and 6-MPH_4 (B). Rats were administered MDMA (10 mg/kg) 3 hr prior to being killed. The K_m and V_{max} values are the means ± SEM derived from double-reciprocal plots for each of five animals. The lines shown are based upon the derived K_m and V_{max} values. The points shown are the mean values of 1/velocity at each substrate concentration. Key: (*) $P < 0.05$ versus saline control.

resulted in enzyme activity decreasing to only 53% of control. Similar results were observed for striatal TPH activity in that citalopram only partially, but significantly ($P<0.001$), antagonized the effect of MDMA on enzyme activity (not shown).

Figure 4B illustrates the cortical 5-HT concentrations from the same experiment. In contrast to the effect of citalopram on the MDMA-induced decrease on TPH activity, the uptake inhibitor completely blocked the decline in 5-HT concentrations produced by MDMA.

Results from the 3-hr experiment with citalopram indicate that the acute effect of MDMA on TPH activity still occurred to some extent. We have reported that there is a recovery of 5-HT synthesis at 1 week when another 5-HT uptake inhibitor, fluoxetine, is coadministered with MDMA [3]. This suggests that a recovery of TPH activity can occur if the onset of MDMA-induced neurotoxicity is blocked. To test this hypothesis, rats were first treated with MDMA (20 mg/kg) at time zero followed by fluoxetine (5 mg/kg) at 3 hr when both cortical TPH activity and 5-HT concentrations would be depressed significantly. Animals were then killed at times corresponding to 3, 6, 12 and 24 hr after MDMA. In a separate experiment, a similarly treated group was allowed to survive for 7 days.

Figure 5 illustrates results from these experiments. As already described, MDMA produced a rapid fall in TPH activity to approximately 40% of control at 3 hr when fluoxetine was administered. Over the next three time points, the single fluoxetine treatment did not alter markedly the effect of MDMA on cortical TPH activity, although it did appear to reduce the degree to which enzyme activity fell. However, by 7 days enzyme activity had returned to control levels in animals treated with fluoxetine compared to 54% of control for animals receiving MDMA alone.

Effects of various drug treatments on the MDMA-induced depression of 5-HT synthesis. The structural similarities between MDMA and methamphetamine suggest a potential role for neurotransmitter release in the effects of MDMA on serotonergic neurons. The role of dopamine release was examined in experiments in which rats were pretreated with the tyrosine hydroxylase inhibitor, alpha-methyl-*p*-tyrosine (100 mg/kg), 1 hr prior to the administration of MDMA. The animals were killed 3 hr following

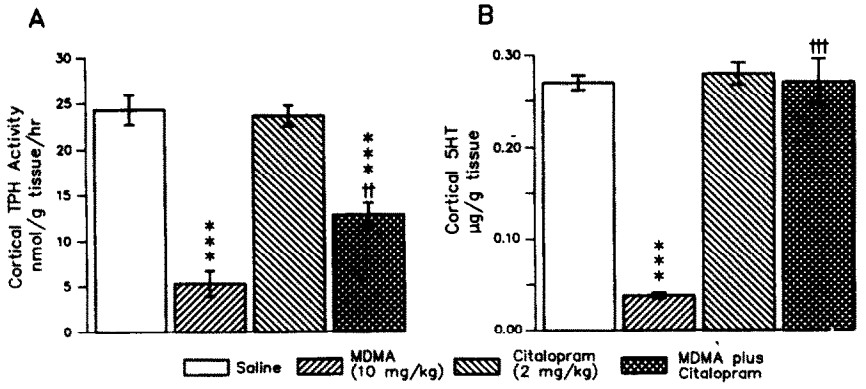

Fig. 4. Effect of the 5-HT uptake inhibitor, citalopram, on the MDMA-induced depression of cortical TPH activity (A) and 5-HT concentrations (B) at 3 hr. Citalopram (2 mg/kg) was administered simultaneously with MDMA (10 mg/kg). Each value is the mean ± SEM for five animals. Key: (***) $P < 0.001$ versus saline control; (††) $P < 0.01$ and (†††) $P < 0.001$ versus MDMA alone.

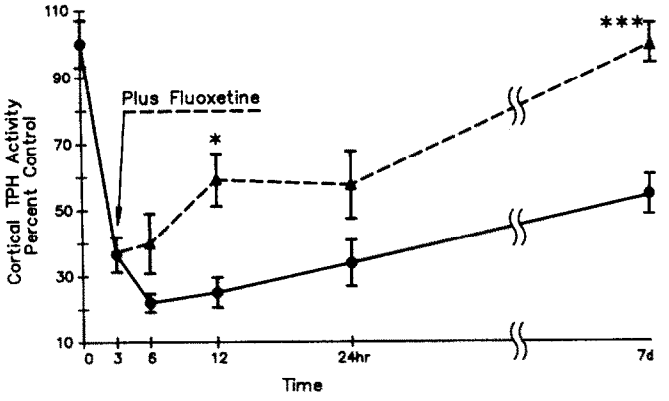

Fig. 5. Effect of fluoxetine on the recovery of cortical TPH activity after MDMA administration. Fluoxetine (5 mg/kg) was given 3 hr following MDMA (20 mg/kg). Enzyme activity in saline controls was 35.6 ± 2.5 nmol oxidized/g tissue/hr. Each value is the mean ± SEM for five animals. Key: (*) $P < 0.05$ and (***) $P < 0.001$ versus MDMA alone.

MDMA, and cortical TPH activity and 5-HT concentrations were determined. Pretreatment with alpha-methyl-*p*-tyrosine did not alter the depression of either TPH activity (Table 2) or 5-HT concentrations (data not shown) produced by MDMA administration.

To obtain a more pronounced depletion of catecholamine stores, as well as to also reduce 5-HT levels, rats were pretreated with reserpine (5 mg/kg, s.c.) 18 hr prior to MDMA administration; animals were then killed 3 hr after MDMA. Reserpine reduced cortical 5-HT concentrations in these animals to less than 5% of control values (not shown). Although 5-HT concentrations were greatly reduced, MDMA still depressed cortical TPH activity to the same extent as that measured in non-reserpinized animals (Table 2).

The effects of 5-HT receptor antagonists on the MDMA-induced changes were examined next. Coadministration of the selective 5-HT_2 antagonist, ketanserine (2.5 mg/kg, i.p.), had a small and inconsistent effect on the depletion of cortical 5-HT by MDMA but, in duplicate experiments, partially blocked the MDMA-induced depression of cortical TPH activity (Fig. 6A). Similar results were observed with the putative 5-HT autoreceptor antagonist, methiothepin (15 mg/kg, i.p.), except that the antagonist slightly reduced the depletion of neurotransmitter produced by MDMA as well as partially blocked the loss of TPH activity produced by MDMA (Fig. 6B). The effects of both antagonists were similar in that MDMA alone reduced cortical TPH activity to approximately 30% of control, while coadministration of ketanserine or methiothepin with MDMA maintained enzyme activity at 55–65% of control.

Noradrenergic input to serotonergic neurons is known to alter TPH activity by activation of alpha-adrenergic receptors. The alpha-2 agonist, clonidine, has been shown to reduce both TPH activity and

Table 2. Effect of pretreatment with α-methyl-*p*-tyrosine, reserpine or yohimbine on the loss of cortical TPH activity 3 hr after MDMA

Treatment	Cortical TPH activity (nmol/g tissue/hr)	% Control
Saline	39.7 ± 3.4 (10)	100 ± 8.6
MDMA (10 mg/kg)	14.9 ± 2.4*** (10)	37.5 ± 6.1
α-Methyl-*p*-tyrosine (100 mg/kg)	41.0 ± 5.6 (5)	103 ± 14.1
MDMA plus α-methyl-*p*-tyrosine	19.9 ± 2.9 (5)	50.1 ± 7.3
Reserpine (5 mg/kg)	39.8 ± 3.7 (5)	100 ± 9.3
MDMA plus reserpine	10.2 ± 4.0 (5)	25.7 ± 10.1
Yohimbine (2 mg/kg)	35.3 ± 1.7 (5)	88.9 ± 4.3
MDMA plus yohimbine	17.4 ± 0.7 (5)	43.8 ± 1.8

α-Methyl-*p*-tyrosine and yohimbine were given 1 hr prior to MDMA. Reserpine was administered 18 hr prior to MDMA. N is given in parentheses.
(***) $P < 0.001$ versus saline control.

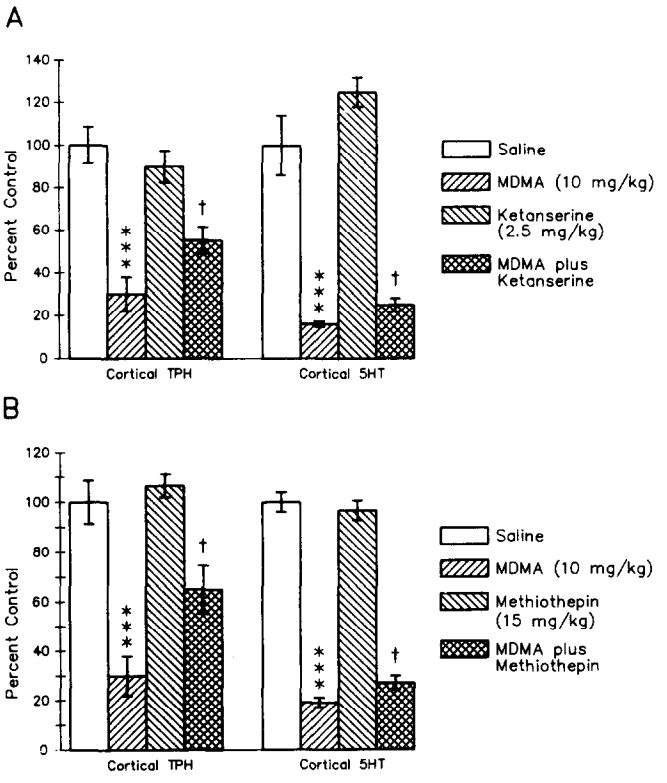

Fig. 6. Effect of coadministration of the 5-HT receptor antagonists ketanserine (A) or methiothepin (B) on the MDMA-induced depression of cortical TPH activity and 5-HT concentrations at 3 hr. Control enzyme activity and 5-HT concentrations were 47.8 ± 4.1 nmol oxidized/g tissue/hr and 0.36 ± 0.01 μg/g tissue respectively. Each value is the mean ± SEM for five animals. Key: (***) $P < 0.001$ versus saline control; and (†) $P < 0.05$ versus MDMA alone.

5-HT synthesis in the rat brain [11]. The selective alpha-2 antagonist, yohimbine, was therefore administered with MDMA to determine if such a mechanism might be involved in the acute depression of TPH activity observed here. Yohimbine (2 mg/kg, i.p.) was administered 1 hr prior to MDMA (10 mg/kg), and the animals were killed at 3 hr. As shown in Table 2, pretreatment with the alpha-antagonist did not alter the MDMA-induced change in cortical TPH activity nor were changes in cortical 5-HT concentrations affected by the presence of yohimbine (not shown). MDMA alone reduced cortical TPH activity and 5-HT concentrations to 38 and 18% of control, respectively, while the same values were 44 and 21% in yohimbine-pretreated animals.

DISCUSSION

The results of this study demonstrate that acute administration of the psychotomimetic agent MDMA produced a rapid decrease in the activity of the rate-limiting enzyme for 5-HT synthesis in the rat brain. Our results indicate that inhibition of transmitter synthesis and increased release combine to produce the dramatic reduction in 5-HT concentrations after MDMA. The requirement for activation of carrier-mediated release in the rapid depletion of 5-HT by MDMA was demonstrated by the effect of uptake blockade on TPH activity compared to 5-HT concentrations. Coadministration of MDMA and the 5-HT uptake inhibitor, citalopram, completely blocked the effect of MDMA on cortical (Fig. 4) and striatal 5-HT concentrations [2]. However, uptake inhibition only partially blocked the effect of MDMA on TPH activity (Fig. 4). This indicates that a large part of the effect of citalopram on 5-HT concentrations following MDMA treatment is due to its interference with the carrier-mediated efflux of the transmitter from the nerve terminal and is not due to protection of TPH activity. The results also support the contention that the uptake carrier is not critical for the transport of MDMA into the nerve terminal as previously demonstrated in synaptosomal uptake experiments using [^{3}H]MDMA [2]. That MDMA is still able to partially reduce TPH activity in the presence of citalopram indicates that the drug still has access to the interior of the neuron.

It is apparent that MDMA administration can trigger some unique change in the serotonergic neuron which results in the loss of TPH activity. This is not a direct action of the drug on the enzyme as shown by the lack of effect of MDMA on TPH activity *in vitro* (Table 1). The results with piperonyl butoxide also indicate that metabolism to MDA is not required. TPH is an allosterically regulated enzyme that can be phosphorylated by calcium–

calmodulin-dependent protein kinase with a resultant increase in affinity for its hydroxylase cofactor [for review see Ref. 14]. Our results show no change in affinity of the cortical enzyme for either the semisynthetic cofactor 6-MPH_4 or tryptophan 3 hr after MDMA administration to rats. This would suggest that the mechanism of enzyme inactivation after MDMA treatment does not involve enzyme dephosphorylation. Weekley *et al.* [11] have described similar changes in TPH activity after *in vivo* administration of the alpha$_2$-agonist, clonidine. The effect of clonidine was biphasic with an increase in enzyme activity at 25 min followed by a decrease in TPH activity at 90 min (measured *ex vivo*) through an apparent reduction in the V_{max} of the enzyme. This effect was reportedly reversible, which seems to differentiate it from the effect of MDMA on enzyme activity.

Although the results of Weekley *et al.* [11] suggest that adrenergic mechanisms can initiate changes in TPH activity qualitatively similar to those described here, our results with alpha-methyl-*p*-tyrosine and yohimbine do not support a role for catecholamines in the effect of MDMA on TPH activity. The dose of yohimbine used here has been shown to be sufficient to antagonize alpha-agonist-induced inhibition of norepinephrine turnover [15], but it did not alter the effect of MDMA on TPH activity. The dose of alpha-methyl-*p*-tyrosine was selected based upon its ability to cause depletions of striatal dopamine in the range of 60% and to antagonize the reduction of TPH activity caused by methamphetamine [16]. The latter has been shown to require ongoing dopamine synthesis and is also blocked by inhibitors of the 5-HT uptake carrier [16, 17]. The lack of effect of alpha-methyl-*p*-tyrosine pretreatment on the MDMA-induced loss of TPH activity suggests that MDMA may not be acting through the same mechanism as methamphetamine.

Beyond the possible involvement of catecholamines, several other mechanisms were considered as potential initiators in the loss of TPH activity after MDMA administration. Since MDMA is able to deplete neuronal 5-HT by up to 80% within 3 hr of drug administration, it is apparent the drug can displace intraneuronal stores of the transmitter which are then free to be transported out of the neuron by the 5-HT uptake carrier. To determine if the loss of TPH activity was somehow triggered by an increase in cytoplasmic concentrations of neurotransmitter, 5-HT concentrations were first depleted by 95% with reserpine and then MDMA was administered. MDMA still depressed TPH activity in reserpinized animals. Since the dose of reserpine used here would also have produced a significant depletion of catecholamines, this result may also have bearing on the role of catecholamines in this response, keeping in mind, however, that amphetamines are considered to release transmitter from a reserpine-insensitive pool [18].

The results of experiments conducted with both ketanserine and methiothepin suggest that the effect of MDMA on TPH activity may depend to some extent on the release of transmitter and receptor activation since both receptor antagonists partially blocked the MDMA-induced loss of enzyme activity. The selective 5-HT_2 antagonist, ketanserine, blocks activation of the 5-HT receptor subtype generally believed to mediate the behavioral effects of serotonergic agonists such as quipazine and hallucinogenic agents such as lysergic acid diethylamide and mescaline [19]. The fact that blockade of these receptors partially antagonized the depression of TPH activity after MDMA implies that a trans-synaptic effect may be involved. The effect of methiothepin on the MDMA-induced decrease in TPH activity may have a similar explanation since methiothepin has good affinity for the 5-HT_2 receptor [20]. Additionally, however, methiothepin has been shown to possess affinity for the 5-HT autoreceptor [21] and has been used to block activity at the autoreceptor both *in vivo* [22] and *in vitro* [20]. Like ketanserine, methiothepin reduced the loss of TPH activity from approximately 30% of control with MDMA alone to 60% when the antagonists were also administered. Whether this is an autoreceptor effect or due to 5-HT_2 receptor blockade is unknown. Receptor antagonism would not be expected to influence the release of 5-HT by MDMA, and the data support this. Neither antagonist had a dramatic effect on the decrease in cortical 5-HT concentrations measured 3 hr after MDMA. Citalopram which does block MDMA-induced 5-HT release *in vitro* [2] and *in vivo* would also be expected to partially antagonize the effect of MDMA on TPH activity not by blocking receptors but instead by reducing synaptic levels of the transmitter. Figure 3A shows that citalopram did indeed antagonize the effect of MDMA on TPH activity to approximately the same extent as did the two receptor antagonists. It is worth mentioning that all three drugs did, to varying degrees, antagonize some of the behavioral activation produced by MDMA in the rats, although this was difficult to evaluate for methiothepin which had a strong sedating effect on the animals.

Several pieces of evidence indicate that the acute effects of MDMA on the serotonergic neuron are not permanent. In the presence of 5-HT uptake inhibition, TPH activity was still depressed significantly 3 hr following MDMA administration (Fig. 4); however, 5-HT synthesis is back at control levels by 7 days [3]. Furthermore, an apparently non-neurotoxic analog of MDMA, *N*-ethyl-3,4-methylenedioxyamphetamine, produces the acute phase of serotonin depletion [12] and TPH inhibition (C. J. Schmidt and V. L. Taylor, unreported observation), yet again, there is an apparent recovery of 5-HT synthesis by 1 week. Along the same lines, both stereoisomers of MDMA produced an acute loss of TPH activity (C. J. Schmidt and V. L. Taylor, unreported observation); however, only the (+)-stereoisomer of MDMA produces the long-term depletion of cortical [3] or striatal [2] 5-HT concentrations, suggesting that recovery of TPH activity occurs after the (−)-stereoisomer. Finally, as shown here, administration of fluoxetine 3 hr after MDMA, when TPH activity was reduced to approximately 40% of control, allowed complete recovery of enzyme activity by 7 days. The long time period required for the recovery of enzyme activity suggests that new synthesis of enzyme may be required. Taken together these results indicate that TPH is irre-

versibly inactivated following administration of MDMA or its analogs. Eventual recovery of 5-HT synthesis is dependent upon whether or not the neurotoxic phase of the drug effect develops.

It is worth noting that there may be a pool of TPH which is resistant to the effect of MDMA. In spite of a rather steep dose–response relationship, Fig. 1 shows that increasing the dose of MDMA beyond 10 mg/kg did not reduce TPH activity further. This finding may be related to the recent observation of O'Hearn *et al.* [23] that an anatomically distinct class of 5-HT containing axons in the rat cerebral cortex are apparently resistant to the effects of MDMA.

In summary, although the mechanism responsible for the MDMA-induced inactivation of TPH remains unknown, the effect is apparently not due to a direct action of the drug on TPH. Experiments with 5-HT receptor antagonists indicate that serotonin release from a reserpine resistant pool may be involved in the response. Additional studies are required to further examine this possibility. The acute response of serotonergic neurons and, in particular, TPH to MDMA is unique in its time course, neurochemical basis and possibly its mechanism. Further study of this effect should provide valuable insight into the regulatory mechanisms controlling TPH activity and 5-HT synthesis in the brain.

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