
8. NEUROCHEMICAL EFFECTS OF MDMA

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1. INTRODUCTION

Since 1971 we have extensively investigated the neurochemical effects of amphetamine and related congeners. Early in those studies, we observed that methamphetamine, given in large repeated doses (10–15 mg/kg, s.c., every six hours for five doses), caused a dose-related decrease in tyrosine hydroxylase (TH) activity in the neostriatum [1, 2] and substantia nigra [3]. A parallel decline in concentrations of dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) [4], accompanied the decrease in enzyme activity.

We first suspected that dopamine may be involved in the response to methamphetamine when dopamine antagonists prevented the methamphetamine effects [5, 6]. More convincing evidence for the role of dopamine was obtained when we observed that inhibition of dopamine synthesis with α -methyl-p-tyrosine (MT), administered concurrently, prevented the methamphetamine-induced decline in tyrosine hydroxylase activity and dopamine content. When the inhibited step in the biosynthesis of dopamine was circumvented by administering L-DOPA and a peripheral decarboxylase inhibitor, the methamphetamine-induced decrease in tyrosine hydroxylase and dopamine content recurred [7].

Additional evidence for the possible role of dopamine in the methamphetamine-induced response was obtained by employing the dopamine uptake inhibitor, amfonelic acid. When amfonelic acid was administered concurrently with methamphetamine, neither tyrosine hydroxylase activity nor dopamine content was compromised [4].

These experiments provided evidence that dopamine is involved in the methamphetamine-induced response. Whether dopamine or a reactive metabolite(s) is responsible for the apparent neurotoxicity associated with methamphetamine administration needs further study.

2. RESULTS AND DISCUSSION

2.1. Methamphetamine effects on the serotonergic and other neurotransmitter systems

We considered whether methamphetamine in large doses caused a generalized effect on all transmitter systems or whether specific systems were selectively affected. The activity of enzymes served as a marker to assess the effect of methamphetamine on these neurotransmitter systems. Repeated large doses of methamphetamine did not alter neostriatal acetylcholinesterase nor glutamic acid decarboxylase activity, which suggested that neither the cholinergic nor the GABAergic systems, respectively, were adversely affected by methamphetamine [8].

In many of the brain regions examined, tryptophan hydroxylase (TPH) activity and concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were decreased rapidly after a single dose of methamphetamine [9,10]. When only one dose was administered, the serotonergic parameters returned to normal within two weeks. However, when repeated doses of methamphetamine were administered, the effects of methamphetamine were more pronounced and persisted for as long as 110 days after the fifth and final dose of the drug [11]. The rate and extent of recovery varied for each brain region.

We characterized further the effects of methamphetamine on the serotonergic system by determining whether agents that prevent or attenuate the response in the dopaminergic systems had a similar protective effect in the serotonergic system. As in the dopaminergic system, haloperidol blocked the effect of repeated doses of methamphetamine on the serotonergic system [9]. Surprisingly, when dopamine synthesis was inhibited with MT, methamphetamine had no effect on neostriatal or hippocampal TPH activity [9]. When synthesis of dopamine was reinstated by administering L-DOPA and a peripheral decarboxylase inhibitor, the decline in TPH activity and content of 5-HT and 5-HIAA returned to control levels [12]. These data suggested that dopamine is essential for the methamphetamine-induced neurotoxicity in the serotonergic system.

Involvement of dopamine in the serotonergic response was further defined by disrupting innervation to a specific brain area and determining whether that dopamine-depleted area, but not regions with intact dopaminergic input, was selectively protected from the methamphetamine [13]. 6-Hydroxydopamine (6-OH-DA) was injected bilaterally into the substantia nigra and methamphetamine was administered 10–14 days later. The decline in neostriatal TPH activity observed in the nonlesioned rat after methamphetamine, was

prevented in the 6OHDA-lesioned neostriatum. In the frontal cortex, however, the decrease in enzyme activity persisted, while in the hippocampus there was an attenuation of the methamphetamine effect. We concluded that dopaminergic innervation is essential for the neurotoxicity in the serotonergic system.

The methamphetamine-induced decrease in TPH activity was prevented by a dopamine uptake inhibitor, amfonelic acid [4]. Moreover an inhibitor of 5-HT uptake, fluoxetine [9, 14], was also effective in blocking the methamphetamine-induced decrease in TPH activity.

In summary, methamphetamine compromises both the dopaminergic and serotonergic systems. Because the effects of multiple doses of methamphetamine persist long after the drug administration is discontinued, we suggest that methamphetamine given in large doses is neurotoxic to both the dopaminergic and serotonergic systems. Histological changes [15, 16], as well as impairment of DA uptake [17] by these drugs, provide additional evidence of a neurotoxic response. It appears that dopamine and/or its reactive metabolite(s), may be responsible for the neurotoxic response in both systems.

2.2. Studies with designer drugs

Seiden and his coworkers [18] reported that 3,4-methylenedioxymphetamine (MDA) caused a long-lasting decrease in rat neostriatal 5-HT uptake and in 5HT and 5-HIAA content; alterations of Fink-Heimer staining suggested that nerve terminal degeneration occurred.

We investigated the effects of 3,4-methylenedioxymphetamine (MDMA) or MDA on dopaminergic and serotonergic systems, by employing techniques previously used in our laboratory to determine alterations of the two transmitter systems after methamphetamine. Three hours after a single injection of either drug (10 mg/kg), neostriatal TPH activity was markedly decreased (Figure 1, Ref. 19). MDMA also reduced enzyme activity in the hippocampus (52% of control) and cerebral cortex (30% of control). Similar responses were observed for MDA. In contrast to methamphetamine, tyrosine hydroxylase activity was unaltered by either drug. MDMA or MDA also caused decreases in 5-HT and 5-HIAA concentrations in the three brain areas (Figure 2). When repeated doses of either agent (10 mg/kg) were administered and rats were sacrificed 18 hours after the last dose, the decrease in TPH activity was further depressed as were the concentrations of 5-HT and 5-HIAA in the three brain regions (data not shown).

In rats that received a single dose of MDMA or MDA, neostriatal dopamine concentrations were elevated to approximately 140% of control; MDMA increased homovanillic acid and MDA decreased dihydroxyphenylacetic acid concentrations (data not shown). It is interesting that although tyrosine hydroxylase activity was not altered, there were transient changes of dopamine and its metabolites. These observations provide evidence that although MDMA and MDA may not cause neurotoxicity in the dopamine system as

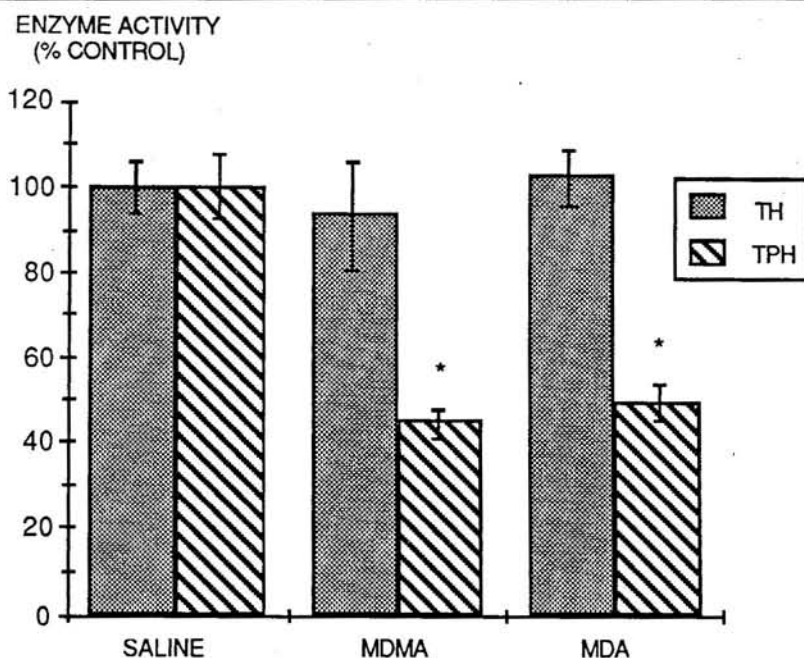


Figure 1. Effect of acute drug treatments on neostriatal tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) activities. Rats were killed 3 hours after a single 10 mg/kg injection of MDA or MDMA. Results are presented as the means $\pm$ SEM ($n=6$) and expressed as percent control. Control values for TH and TPH activities were 2178.8 and 38.2 nmol/g tissue per hour, respectively. * $P < 0.001$ versus control, by the two-tailed Student's t -test. (After Stone et al. [19]. Courtesy Eur. J. Pharmacol.)

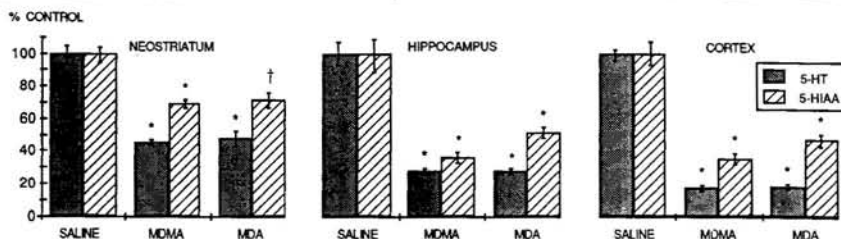


Figure 2. Effect of acute drug treatments on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations. Experimental conditions are described in Figure 1. The means $\pm$ SEM from 6 animals are presented as percent of control. Control values (in $\mu\text{g/g}$ tissue), with 5-HT concentration listed first, were: neostriatum, 0.421 and 0.517; hippocampus, 0.277 and 0.375; cortex, 0.487 and 0.267. * $P < 0.001$, * $P < 0.005$ versus corresponding control, by the two-tailed Student's t -test (After Stone et al. [19]. Courtesy Eur. J. Pharmacol.)

defined by decreases in tyrosine hydroxylase activity, there is, however, a significant effect on dopamine metabolism, as evidenced by the alteration of the content of dopamine and its metabolites.

These responses to MDMA and MDA are strikingly similar to those observed after another amphetamine analogue, *p*-chloroamphetamine [20–24]. After a single dose of *p*-chloroamphetamine, it was discovered by Sanders-Bush et al. [22] that decreases in 5-HT and 5-HIAA concentrations occurred, which persisted for at least four months; TPH activity was also decreased after *p*-chloroamphetamine.

2.3. Isomers of MDMA and MDA

We [25] compared the effects of the two isomers of MDMA and MDA on brain serotonergic parameters. Three doses (3.5, 5.0, or 10.0 mg/kg, every six hours for five administrations) of either isomer were administered; TPH activity and 5-HT and 5-HIAA content were determined in the neostriatum, hippocampus, and frontal cortex. Both isomers of each drug caused qualitatively similar but quantitatively different responses. The *d*-isomer was more potent than the *l*-isomer of MDMA, at both the 5 and 10 mg/kg dose, in decreasing TPH activity in all three brain areas (Figure 3). There was no quantitative difference between the effects of the *d*- and *l*-isomers of MDA on TPH activity.

There were parallel quantitative differences in the effects of the two isomers of MDMA on 5-HT and 5-HIAA concentrations. The *d*-isomer was more potent than the *l*-isomer of MDMA at the two higher doses in all three brain regions (data not shown). In those areas where there was a quantitative difference in the decrease of 5-HT and 5-HIAA concentrations caused by 3.5 mg/kg of MDA, the *d*-isomer was more potent than the *l*-isomer. At higher doses, the isomers of MDA were equipotent.

In summary, when quantitative differences do occur, the *d*-isomer of MDMA is more potent than the *l*-isomer.

2.4. *N*-ethyl-3,4-methylenedioxyamphetamine

We were interested as to how the response of other congeners of MDMA might differ from that observed for the parent compound. Like MDMA and MDA, the *N*-ethylated derivative of MDA, *N*-ethyl-3,4-methylenedioxyamphetamine (MDE) decreased TPH activity and lowered concentrations of 5-HT and 5-HIAA in the various brain areas; moreover, the *N*-ethylated analog did not alter tyrosine hydroxylase activity [26, 27]. Interestingly, MDE was much less potent than MDMA or MDA. Three hours after a single dose of MDE, neostriatal TPH activity was decreased to approximately 70% of control (Figure 4); neostriatal enzyme activity three hours after MDMA was normally depressed to approximately 45% of control (data not shown).

The rate of recovery of TPH activity in concentrations of 5-HT and 5-HIAA, after multiple doses of MDE, was more rapid than after MDMA or

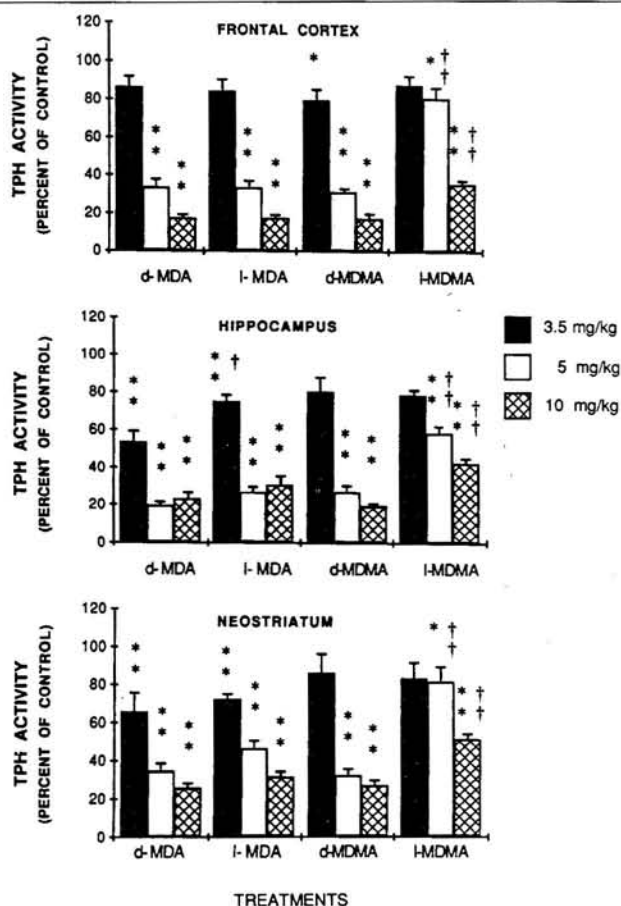


Figure 3. Effects of MDA and MDMA isomers on TPH activities within frontal cortex, hippocampus, and neostriatum. Isomers of MDMA or MDA (3.5, 5, or 10 mg/kg s.c.) were administered for 5 doses at 6-hour intervals, and animals were killed 18 hours later. Results are expressed in percentage of control values (saline treatment) and represent means $\pm$ SEM of 6–18 rats/group. Actual control values for the 10 mg/kg treatment follow: frontal cortex, 66.5 ± 1.9 nmol tryptophan oxidized/hr/g tissue; hippocampus, 54.5 ± 2.9 nmol tryptophan oxidized/hr/g tissue; neostriatum, 49.5 ± 2.4 nmol tryptophan oxidized/hr/g tissue. * $P < .05$, ** $P < .01$ versus respective control; † $P < .05$, ** $P < .01$ versus corresponding d isomer group. (After Johnson et al. [25]. Courtesy J. Pharmacol. Exp. Ther.)

MDA [26, 27]. With MDE there was significant recovery within 18 hours after the last of five doses of the drug (Figure 5); however, there was no evidence of recovery 18 hours after multiple doses of MDMA or MDA (data not shown). MDE is less potent and the effects are more short-lived than for MDMA and MDA. It is interesting that MDE is less potent than MDMA or MDA in releasing dopamine (28).

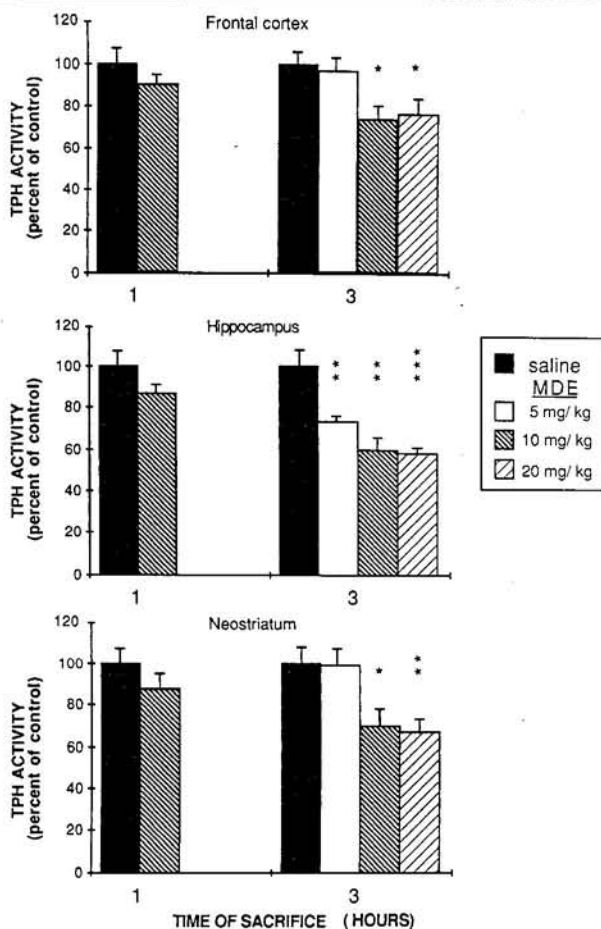


Figure 4. Effects of MDE (10 mg/kg) 1 hour after a single injection and of MDE (5, 10, or 20 mg/kg) 3 hours after the injection on the frontal cortex, hippocampal, and neostriatal TPH activity. The enzymatic activities are expressed as percent $\pm$ SEM of the control group (injected with saline) for the respective time of sacrifice. Enzymatic activities of the control groups (3 hours), expressed in nmol of hydroxylated tryptophan/hr/g of tissue, were: 68.0 ± 4.3 in the frontal cortex; 52.0 ± 4.5 in the hippocampus; and 49.5 ± 4.0 in the neostriatum. Statistical analyses of the enzymatic activity between means of the MDE groups and control were performed with Student's t-test. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (N=6). (After Johnson et al. [27]. Courtesy Biochem. Pharmacol.)

2.5. Immediate and long-term effects of MDMA

The immediate and long-term responses of serotonergic parameters in four different brain regions at varying times after a single dose of MDMA are illustrated in Figure 6 [29]. TPH activity declined significantly in the frontal cortex within 15 minutes and in the neostriatum, hippocampus, and hypo-

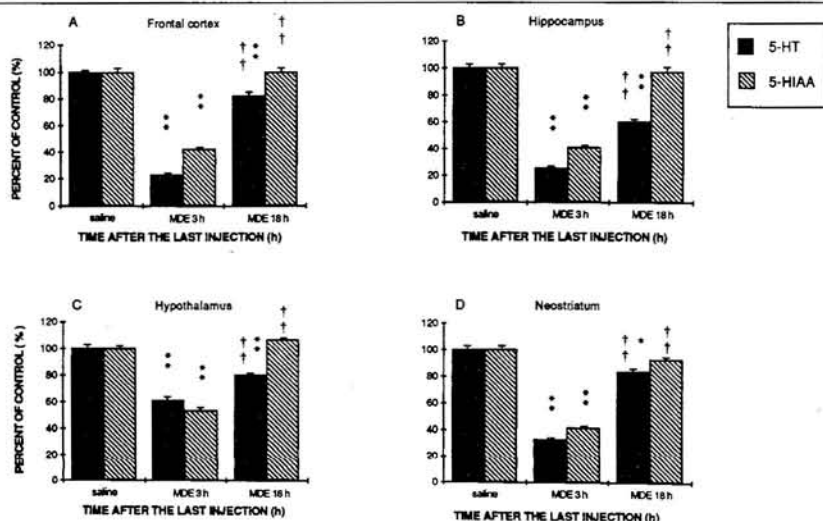


Figure 5. Effects of multiple administrations of MDE (10 mg/kg) on 5-HT and 5-HIAA concentrations 3 and 18 hours after treatment. The means of the 5-HT and 5-HIAA concentrations in the frontal cortex (A), hippocampus (B), hypothalamus (C), and neostriatum (D) are expressed in a percentage of control (saline) $\pm$ SEM. As there was no significant difference between the two control groups at each time of sacrifice, these determinations were combined and expressed as a single control group in order to simplify the figure. Means of 5-HT concentrations of the control (saline) group, expressed as $\mu\text{g/g}$ tissue, were: 0.62 ± 0.01 in the frontal cortex, 0.40 ± 0.02 in the hippocampus, 0.86 ± 0.04 in the hypothalamus, and 0.43 ± 0.02 in the neostriatum. Concentrations of control 5-HIAA were: 0.19 ± 0.01 in the frontal cortex, 0.32 ± 0.02 in the hippocampus, 0.45 ± 0.02 in the hypothalamus, and 0.41 ± 0.02 in neostriatum. Statistical analyses were performed with a one-way ANOVA test, while a Student-Newman-Keuls test was used for the multiple comparisons analysis. Key: * $P < 0.05$, and ** $P < 0.01$ versus respective control, and † $P < 0.01$ versus corresponding 3-hour group ($N = 16-18$ for control, $N = 6$ for the 3-hour group, $N = 13-14$ for the 18-hour group). (After Johnson et al. [27]. Courtesy of Biochem. Pharmacol.)

thalamus within 60 minutes; 5-HT content followed a similar pattern, while 5-HIAA content declined at a slower rate than the other two serotonergic parameters. Within three to six hours after a single dose of MDMA, all parameters had reached their nadir. There was a rebound towards normal in 5-HT and 5-HIAA concentrations between six and 24 hours in all areas. In the hippocampus and frontal cortex, there was a secondary decline in 5-HT concentrations. The recovery of TPH activity in all three brain regions was slower than for the other two serotonergic parameters.

After a single dose of MDMA (10 mg/kg), dopamine was elevated within one hour and persisted at elevated levels for at least three hours; homovanillic acid concentrations were significantly elevated by three hours. In contrast, dihydroxyphenylacetic acid concentrations were immediately decreased, but returned to normal by 24 hours (data not shown).

When assessing the potential neurotoxicity of MDMA, the long-term

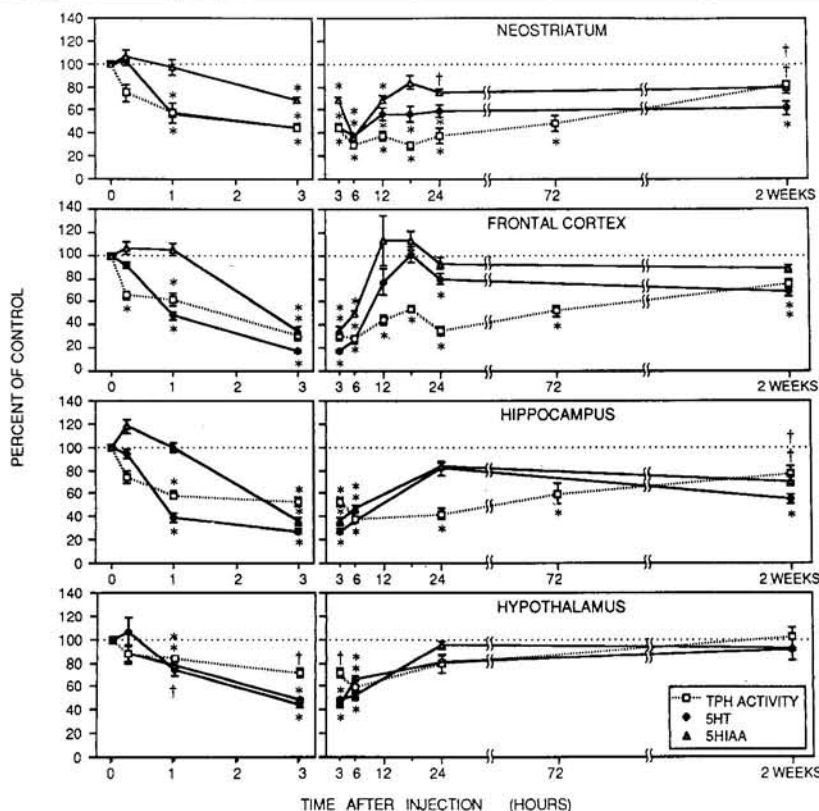
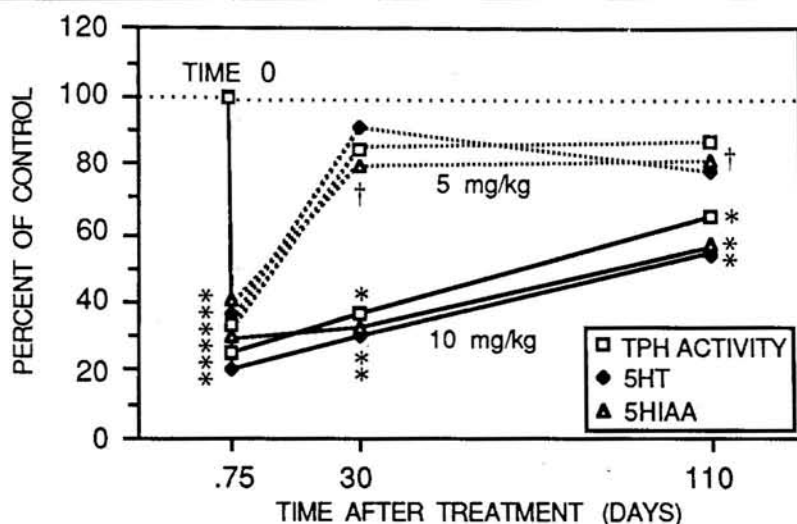


Figure 6. Time course of the regional serotonergic effects of acute administration of MDMA. A single dose of MDMA (10 mg/kg) or saline (control) was injected subcutaneously; rats were killed at specified times thereafter. Each point represents the mean $\pm$ SEM from 4–6 rats, expressed as a percentage of the corresponding control. Immediate effects (up to 3 hours after injection) are represented in the left panel; the right panel diagrams longer-term regional responses (from 3 hour–2 weeks) after injection). One hour control values $\pm$ SEM for neostriata (n), frontal cortex (fc), hippocampus (h), and hypothalamus (ht) were as follows: activity of tryptophan hydroxylase (TPH) (in nmol/g tissue/hr); n = 39.9 ± 3.6 , fc = 80.1 ± 4.9 , h = 63.0 ± 2.6 , ht = 249.3 ± 7.0 ; concentrations of 5-HT and 5-HIAA, respectively (in $\mu\text{g/g}$ tissue): n = 0.533 ± 0.019 and 0.557 ± 0.023 , fc = 0.518 ± 0.018 and 0.218 ± 0.013 , h = 0.359 ± 0.040 and 0.338 ± 0.009 , ht = 0.905 ± 0.064 and 0.396 ± 0.018 . Control values at other times did not vary significantly from those listed above. $^{\dagger}P < 0.05$, $^{*}P < 0.005$ versus corresponding control by the two-tailed Student's *t*-test. (After Stone et al. [29]. Courtesy of Neuropharmacology.)

persisting alterations of serotonergic parameters may be more pertinent than are the transient changes. As can be seen in Figure 6, the effects of a single large dose of MDMA subsided considerably by two weeks. However, when five doses of MDMA (10 mg/kg, given at six-hour intervals) were administered, the decrease in TPH activity persisted in the neostriatum for 110 days (Figure 7). Similar responses were observed in the hippocampus and frontal



† $p < 0.05$, * $p < 0.005$ vs. corresponding saline control

Figure 7. Long-term recovery of serotonergic parameters in the neostriatum after multiple doses of MDMA. Rats were given 5 doses of saline or MDMA (1 dose every 6 hours) and killed at either 18 hours, 30 days, or 110 days after cessation of treatment. Each point represents the mean value (error bars have been omitted for the sake of clarity) from 6–10 rats, expressed as a percentage of the corresponding control. Doses of 5 mg/kg (dotted lines) and 10 mg/kg (solid lines) are represented. Control TPH activity (in nmol/g tissue/hour) at 18 hour, 30 days and 110 days, respectively, was: 45.1 ± 3.5 , 42.8 ± 2.9 , and 45.1 ± 3.6 . Control concentrations of 5-HT and 5-HIAA, respectively, (in $\mu\text{g/g}$ tissue) were: 0.469 ± 0.024 and 0.362 ± 0.023 at 18 hour, 0.365 ± 0.061 and 0.381 ± 0.034 at 30 days; and 0.527 ± 0.047 and 0.453 ± 0.032 at 110 days. † $P < 0.05$ (5-HIAA only), * $P < 0.005$ versus corresponding control, by the two-tailed Student's *t*-test. (After Stone et al. [29]. Courtesy of Neuropharmacology.)

cortex [29]. These persisting serotonergic deficits suggest that MDMA, administered to rats in repeated large doses, is neurotoxic to 5-HT-containing neurons.

2.6. MDMA response in the mouse

Since little is known about the neurochemical effects of MDMA in species other than the rat, we [30] characterized the effects of MDMA in mice, a species that displays a different metabolic profile than that of the rat [31]. The remarkable differences in the time required for the MDMA-induced depressed tryptophan hydroxylase activity to return to normal after a single dose of MDMA in the mouse and in the rat are depicted in Figure 8. As previously reported, the effect in the rat persisted for at least two weeks. In contrast to the rat, however, mouse TPH activity was not significantly altered

ACUTE MDMA IN MOUSE (15 mg/kg) OR RAT (10 mg/kg)

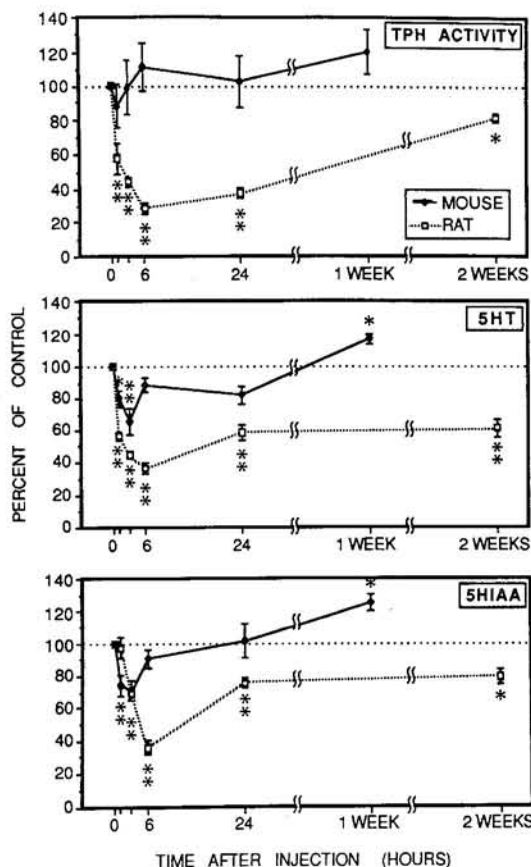


Figure 8. Time course of the neostriatal serotonergic effects of a single dose of MDMA in mouse and rat. MDMA was dissolved in saline and administered as a single subcutaneous injection to mice (15 mg/kg) or rats (10 mg/kg); animals were killed at specified time points thereafter. Points represent means $\pm$ SEM for $n = 6 - 10$ animals, and are expressed as a percent of corresponding time-matched control (vehicle-injected) animals. Representative control values (24-hour time point) for mice and rats, respectively, were: tryptophan hydroxylase (TPH) activity (in nmol/g tissue/hour): 27.0 ± 4.0 and 40.0 ± 2.5 ; 5-hydroxytryptamine (5-HT) concentration (in $\mu\text{g/g}$ tissue): 0.379 ± 0.021 and 0.503 ± 0.042 ; 5-hydroxyindoleacetic acid (5-HIAA) concentration (in $\mu\text{g/g}$ tissue): 0.228 ± 0.021 and 0.430 ± 0.028 . Data were statistically analyzed by a two-way analysis of variance followed by the Student-Newman-Keuls multiple comparisons test. * $P < 0.05$, ** $P < 0.01$ versus corresponding control. (After Stone et al. [29]. Courtesy of Neuropharmacology.)

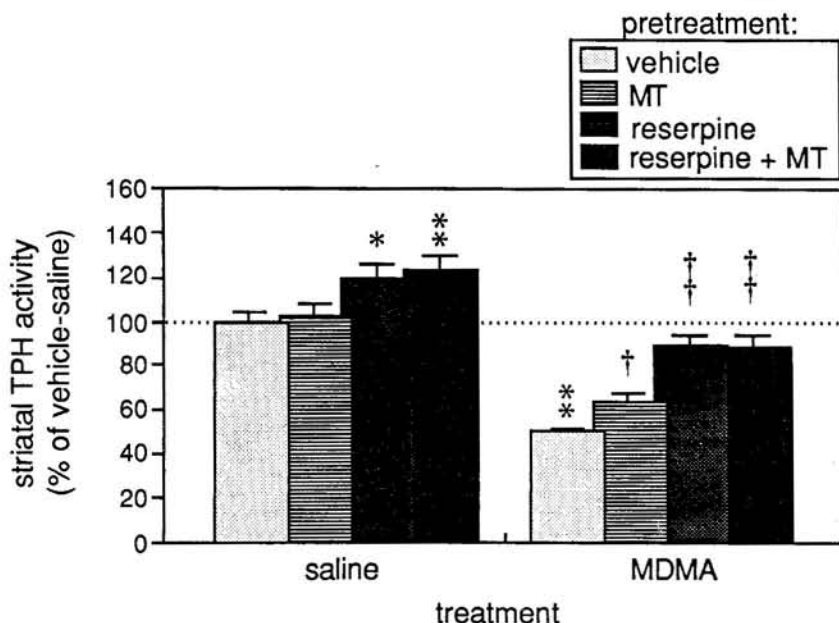


Figure 9. Effect of prior dopamine depletion on the immediate MDMA-induced loss of neostriatal TPH activity. Rats were pretreated with MT (120 mg/kg, i.p.), reserpine (5 mg/kg, i.p.), or reserpine + MT (5 mg/kg and 60 mg/kg, respectively, i.p.) 90 min, 12 hours, or 12 hours + 90 min, respectively, prior to acute MDMA (5 mg/kg, s.c.) or saline (control); animals were killed 3 hours later. Results presented are the means $\pm$ SEM ($n = 6 - 11$), expressed as a percent of control (vehicle-saline). Control value for TPH activity was 49.2 ± 1.9 nmol/g tissue/hour. * $P < .05$, ** $P < .01$ versus vehicle-saline, $^{\dagger}P < .05$, $^{**}P < .01$ versus vehicle-MDMA. Because reserpine pretreatments alone significantly elevated TPH activity, values from MDMA-treated rats were expressed as a percentage $\pm$ SEM of their respective (same pretreatment) saline-treated control mean: TPH activity for the reserpine-MDMA and reserpine + MT-MDMA groups, respectively, were $74.6 \pm 3.4\%$ and $71.7 \pm 4.2\%$ versus $50.3 \pm 1.3\%$ for vehicle-MDMA; pretreatment versus vehicle ($p < 0.01$). (After Stone et al. [33]. Courtesy of J. Pharmacol. Exper. Ther.)

after a single dose of MDMA. Mouse 5-HT and 5-HIAA concentrations were significantly decreased at three hours but had returned to normal within six hours after the MDMA was given.

When multiple larger doses of MDMA were administered to mice at more frequent intervals (six doses, 15 mg/kg, at four-hour intervals), TPH activity was significantly decreased in the neostriatum (60% of control) and hippocampus (35% of control) three hours after the last dose and remained significantly depressed in the hippocampus one week after treatment. Similar responses were observed for 5-HT and 5-HIAA (data not shown).

In summary, at comparable doses, MDMA is less toxic in the mouse than in the rat. These studies provide evidence for the importance of comparing responses to potential neurotoxins in a variety of species in order to assess their risks in humans.

2.7. Role of dopamine in MDMA effects

Because of our previous observations that dopamine is necessary for methamphetamine to cause serotonergic deficits, it was important to determine the possible role of dopamine in the MDMA-induced alterations of the 5-HT system. MDMA is known to elevate dopamine [19, 32], which suggests that dopamine release is enhanced *in vivo* by MDMA. We previously reported that inhibition of dopamine synthesis by MT prevents methamphetamine-induced alterations of both the dopaminergic [7] and the serotonergic system [9, 12]. When dopamine synthesis was reinstated by administering L-DOPA and a peripheral decarboxylase inhibitor, the methamphetamine-induced deficit in the 5-HT system recurred [12].

A similar experiment was conducted with MDMA to determine whether dopamine is essential for the MDMA-induced neurotoxic effects. In these experiments, dopamine concentrations were depleted by MT, reserpine, or with reserpine + MT prior to acute MDMA or saline; animals were sacrificed three hours later (Figure 9). MDMA caused the decrease in neostriatal TPH activity [33]. Prior administration of α -methyl-p-tyrosine (MT) partially prevented the MDMA-induced TPH decline, while reserpine, administered with or without MT, effectively prevented the decline of neostriatal enzyme activity in the MDMA-treated rats.

When multiple doses of MT and MDMA were given concurrently, MT attenuated the longer-term (18 hours) effect of MDMA (5 mg/kg) on TPH and 5-HT content, but did not alter the responses to the higher dose of MDMA (10 mg/kg). In a separate experiment we administered MT 90 minutes before a single larger dose of MDMA (20 mg/kg), and rats were sacrificed three days later. MT attenuated the effects of MDMA on TPH activity and 5-HT content but did not alter the effects of MDMA on 5-HIAA content. Using a paradigm in which reserpine (5 mg/kg) was substituted for MT, reserpine completely prevented the long-term (three-day) response to MDMA, given in high doses (Figure 10) [33]. It is interesting that when the attenuation by MT or reserpine of the response to MDMA and methamphetamine is compared, MT appeared less effective than reserpine in preventing the response to MDMA. This could possibly be attributed to the greater degree of dopamine depletion by reserpine as compared to MT. It is also possible that depletion of 5-HT, in addition to dopamine, by reserpine may also be important in the protection. We are currently conducting experiments to explore these possibilities.

Two additional experiments were conducted to explore the role of dopamine in the serotonergic response to MDMA. We have previously demonstrated that a selective lesion of the dopaminergic input to the neostriatum from the substantia nigra prevented the effects of a single dose of methamphetamine on TPH activity [13]. A similar experiment was conducted with MDMA. Bilateral injections of 60HDA into the substantia nigra were performed seven to ten days before MDMA. Three hours after MDMA was

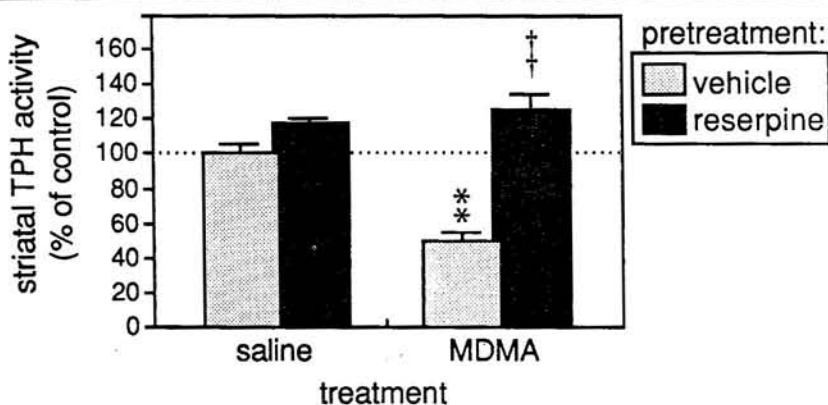


Figure 10. Effect of reserpine pretreatment on the persistent MDMA-induced reduction in striatal TPH activity. Reserpine (5 mg/kg) or vehicle was administered i.p. 12 hours prior to a single dose of MDMA (20 mg/kg, s.c.) or saline; rats were killed 3 days later. Results are represented as the means $\pm$ SEM ($n = 6 - 7$), expressed as a percent of control (vehicle-saline). Control TPH activity was 43.4 ± 2.6 nmol/g tissue/hour. ** $P < .01$ versus vehicle-saline; †† $P < .01$ versus vehicle-MDMA. (After Stone et al. [33]. Courtesy J. Pharmacol. Exp. Ther.)

administered, the rats were killed and TPH activity was compared in the neostriatum, frontal cortex, and hippocampus (Figure 11). In the neostriatum, where there was no longer dopaminergic input, the MDMA-induced decrease in TPH activity was essentially prevented; however, in the frontal cortex and hippocampus, where dopaminergic innervation was intact, MDMA still caused a significant decrease in TPH activity. At the lower dose of MDMA there was some protection in the hippocampus, which is thought to have some dopaminergic innervation from the substantia nigra [34].

We [4] previously reported that amfonelic acid, a dopamine uptake blocker, attenuated the effects of methamphetamine on the 5-HT system. In a similar fashion, we (Figure 12) [33] investigated the effects of another specific dopamine uptake inhibitor, GBR 12909, on the MDMA response. GBR 12909 (20 mg/kg) was administered 15 minutes prior to a single dose of MDMA (20 mg/kg); rats were killed three days later. The dopamine uptake inhibitor effectively attenuated the MDMA-induced decrease in TPH activity and in 5-HT and 5-HIAA content.

The above experiments provide convincing evidence that dopamine plays a role in the MDMA-induced changes in the serotonergic system. When dopamine was depleted with MT or reserpine, the MDMA effects were attenuated. Moreover, when dopaminergic input was disrupted by lesioning the nigrostriatal pathway with 6OHDA, the response to MDMA was attenuated. Finally, when dopamine uptake was blocked with GBR 12909, MDMA again was less effective in eliciting long-term serotonergic deficits.

If dopamine is involved with the MDMA and methamphetamine-induced

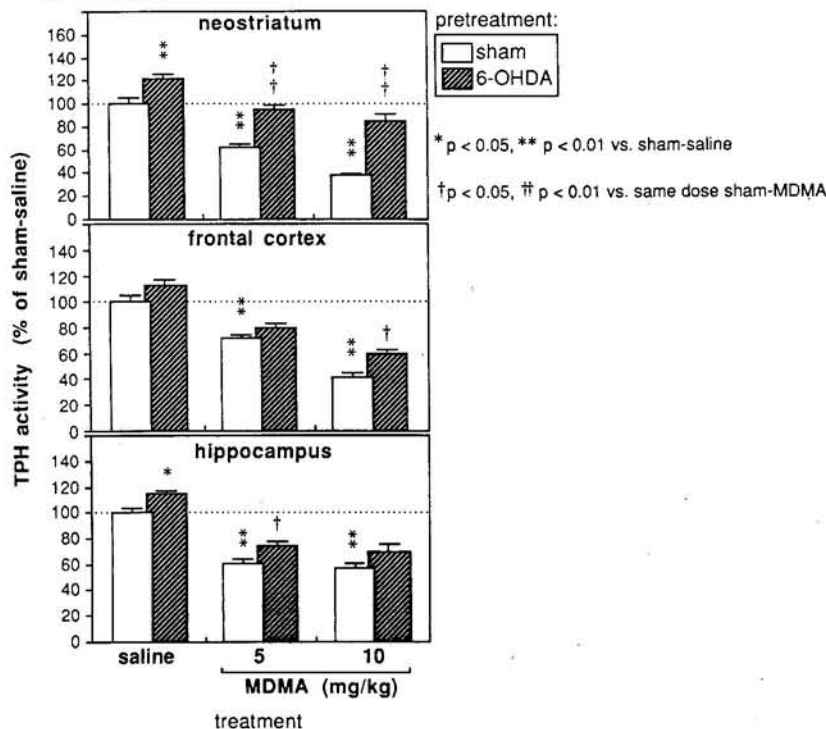


Figure 11. Effect of prior substantia nigral lesions on the immediate MDMA-induced decreases in regional TPH activity. Lesions were induced bilaterally by local injection of 4 μ g 6OHDA/8 μ l 0.1% ascorbate saline/side. Control rats received sham lesions of ascorbate vehicle alone. Following a 7–10 day recovery period, acute MDMA (5 or 10 mg/kg) was administered s.c. and rats were killed 3 hours later. Results are the means $\pm$ SEM, expressed as a percent of sham-saline ($n = 22$ for sham-saline group, $n = 14$ for 6OHDA-saline group, $n = 6 - 12$ for MDMA-treated groups). Control TPH activities (in nmol/g tissue/hour) were: striatum, 42.2 ± 2.3 ; frontal cortex, 77.3 ± 3.7 ; hippocampus, 52.2 ± 1.8 . * $P < .05$, ** $P < .01$ versus sham-saline, † $P < .05$, †† $P < .01$ versus corresponding sham-MDMA. By 2-way ANOVA and Newman Keuls multiple comparisons test. Because 6OHDA itself significantly elevated TPH activity, values from MDMA-treated rats were expressed as percentage $\pm$ SEM of their respective saline-treated control mean: in the neostriatum, TPH activity for the 6OHDA-MDMA group was $67.6 \pm 5.1\%$ versus $37.5 \pm 2.3\%$ for sham-MDMA, $P < 0.01$ by Student's t -test. When similarly expressed, no significant differences were found between sham-MDMA and 6OHDA-MDMA groups in the hippocampus or frontal cortex. (After Stone et al. [33]. Courtesy of J. Pharmacol. Exp. Ther.)

neurochemical effects as we suggest, the exact mechanism remains elusive. It is known that these drugs release dopamine and that dopamine can be readily oxidized to reactive metabolites that could possibly destroy nerve terminals [35, 36]. Moreover, inhibition of MAO by these drugs [37] could enhance this response. The possibility that 6OHDA is formed resulting in destruction of nerve terminals, as suggested by Seiden et al. [38], also is an important consideration.

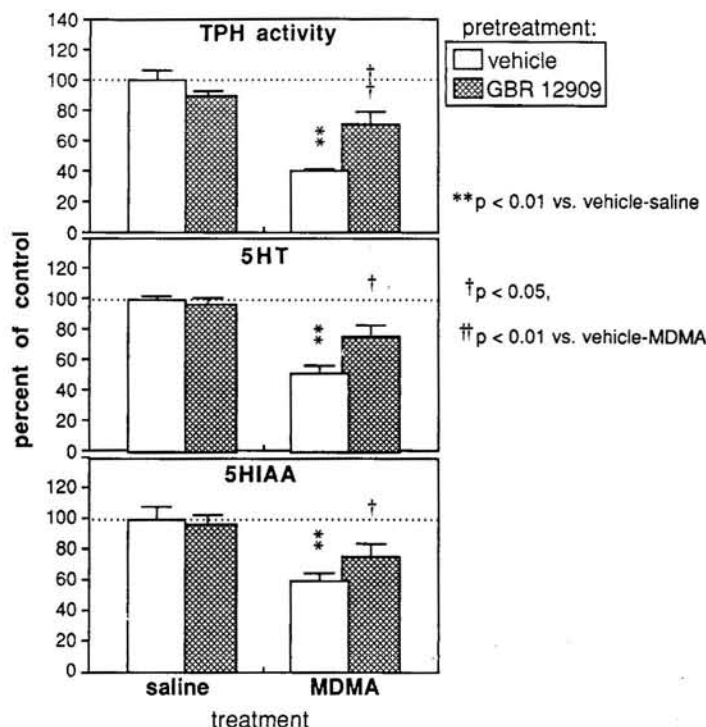


Figure 12. Effect of dopamine-uptake inhibition on the toxic serotonergic deficits induced by acute MDMA. GBR 12909 (20 mg/kg, i.p.) or vehicle was administered 15 minutes prior to a single dose of MDMA (20 mg/kg, s.c.); rats were killed 3 days later. Results depicted are the means $\pm$ SEM ($n = 5 - 6$), expressed as a percent of control (vehicle-saline). Control values were: TPH activity (in nmol/g tissue/hour), 53.7 ± 3.1 ; 5-HT and 5-HIAA (in $\mu\text{g/g}$ tissue), 0.533 ± 0.013 and 0.502 ± 0.040 , respectively. ** $P < .01$ versus vehicle-saline; † $P < .05$, †† $P < .01$ versus vehicle-MDMA. (After Stone et al. [33]. Courtesy of Pharmacol. Exp. Ther.)

3. CONCLUSIONS

We have observed that the dopaminergic and serotonergic systems are dramatically altered by methamphetamine. The response of these transmitter systems to the methylenedioxy-derivatives of methamphetamine have been compared. Although MDMA perturbs both the dopaminergic and serotonergic systems, the serotonergic, but not the dopaminergic, system is persistently altered. We have provided evidence that dopamine plays a role in the changes in the serotonergic system induced by both methamphetamine and MDMA.

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