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Direct central effects of acute methylenedioxymethamphetamine on serotonergic neurons

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Acute peripheral administration of either the (+) or (−) stereoisomer of methylenedioxymethamphetamine (MDMA) to rats results in a rapid loss of tryptophan hydroxylase (TPH) activity in several brain regions. This decline in enzyme activity precedes a decrease in serotonin (5-HT) concentrations in the same areas. An initial rise in the concentration of 5-hydroxyindole acetic acid after drug administration suggests that an increase in the turnover of 5-HT is an early event in the development of these changes. Unsuccessful attempts to reproduce the in vivo effects of MDMA on TPH activity using in vitro preparations such as cortical slices or the mouse mastocytoma cell line, P-815, suggested a requirement for an intact neuronal system or metabolism of the drug. Injection of MDMA directly into several brain regions also had no effect on TPH activity or 5-HT concentrations. However, when brain concentrations of MDMA were maintained using a constant i.c.v. infusion, TPH activity declined as observed following peripheral administration. The results, therefore, indicate that the acute effect of MDMA on 5-HT synthesis is a direct central effect of the drug which may be triggered by a sustained increase in transmitter turnover.

3,4-Methylenedioxymethamphetamine (MDMA); Serotonin (5-HT); Tryptophan hydroxylase; Amphetamine; (Neurotoxicity)

1. Introduction

The psychedelic amphetamine analog, methylenedioxymethamphetamine (MDMA), has recently become the subject of considerable research interest. This is in part due to the drug's unique psychoactive properties and the resultant widespread abuse of MDMA. The observation that the desmethyl analog of MDMA, methylenedioxyamphetamine (MDA), was toxic to serotonergic neurons (Ricaurte et al., 1985) in the rat brain as well as the neurotoxicity of the related compound, methamphetamine (Hotchkiss and Gibb, 1980; Schmidt et al., 1985), prompted similar studies on the neurochemical effects of MDMA.

Results from a number of laboratories have demonstrated that MDMA, like MDA, is toxic to serotonergic neurons of the rat brain following either single (Schmidt et al., 1986; Schmidt, 1987a,b; Schmidt et al., 1987) or multiple administration (Stone et al., 1986; Commins et al., 1987; Battaglia et al., 1987). Interestingly, MDMA-induced neurotoxicity appears to differ from that produced by high doses of methamphetamine which affects both the serotonergic and dopaminergic systems of the rat brain (Hotchkiss and Gibb, 1980). Indeed, as suggested previously (Schmidt et al., 1987), the neurochemical profile of MDMA indicates a stronger similarity to the well-known serotonergic neurotoxin, p-chloroamphetamine (PCA). Unfortunately, despite the extensive study of PCA, the mechanism of its neurotoxicity has also yet to be elucidated (Fuller, 1985).

In addition to their neurotoxicity, both PCA (Fuller et al., 1975; Ross, 1976; Sanders-Bush and Steranka, 1978) and MDMA (Schmidt, 1987a, Schmidt et al., 1987) produce a rapid, acute deple-

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tion of serotonin (5-HT) in the rat brain which appears to be independent of the long-term damage to the neurons. In the case of MDMA, this acute depletion of 5-HT has been shown to be due to both a rapid inactivation of tryptophan hydroxylase (TPH) and the carrier-mediated release of 5-HT from the neuron (Schmidt and Taylor, in press). These alterations occur at MDMA and PCA doses well below those required to produce neurotoxicity and, hence, represent a unique pharmacological activity. The mechanism actually responsible for the loss of TPH activity after either drug is unknown, however. The experiments described in this report further characterize the acute effect of MDMA on TPH activity in the rat brain.

2. Materials and methods

2.1. Drug administration

Male Sprague-Dawley rats (200-250 g unless noted otherwise) were maintained on a 12 h light-dark cycle with free access to food and water. MDMA was administered as the free base in saline. All animals were sacrificed by decapitation and the brains were rapidly dissected on ice. The area of the brainstem raphe nuclei was taken by making a coronal cut between the colliculi to the rostral end of the pons. Another cut was made through the brainstem at the distal end of the fourth ventricle. The inferior colliculi were then removed by a horizontal cut. All tissues were stored at $-80\,^{\circ}C$ until assayed.

MDMA was synthesized as the hydrochloride salt according to Braun et al. (1980) as described previously (Schmidt et al., 1987a). The resolved enantiomers of MDMA were provided by the National Institute on Drug Abuse (NIDA).

2.2. Determination of tryptophan hydroxylase activity and indolamine concentrations

TPH activity was assayed by the coupled $^{14}CO_2$ trapping assay described previously (Hotchkiss et al., 1979; Schmidt and Taylor, in press) with the exception that aromatic L-amino acid decarboxylase was partially purified from bovine kidney as described by Lovenberg and Engelman (1971). Radioactivity was determined on a Beckman LS 5801 scintillation counter with quench correction; TPH activity is expressed as nmoles of tryptophan oxidized per g tissue or per mg protein for tissue slices or synaptosomes, respectively. Protein was assayed by the method of Bradford (1976). All reagents were from Sigma Chemical Co. (St. Louis, MO). [1-^{14}C]Tryptophan (50 mCi/mmol) was purchased from New England Nuclear (Boston, MA).

Brain concentrations of 5-HT and its metabolite 5-hydroxyindolacetic acid (5-HIAA) were determined by high performance liquid chromatography with electrochemical detection as described previously (Schmidt, 1987a). For striatal tissues, the concentrations of dopamine and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were also determined (Schmidt et al., 1986).

2.3. Superfusion of cortical slices

Cortical slices (0.25×0.25 mm) were prepared on a McIlwain tissue chopper, washed once in a Krebs-Ringer bicarbonate buffer (KRB) and divided among eight chambers of a superfusion system described previously (Schmidt and Gibb, 1985). The slices were superfused for 2 h with KRB containing 10 μM tryptophan. Four of the chambers were superfused with KRB containing 250 μM MDMA while four chambers functioned as controls. At the termination of the experiment the slices were removed from the chambers, pelleted at $1000 \times g$ and frozen at $-80\,^{\circ}C$ until assayed for TPH activity.

2.4. P-815 cultures

The murine mastocytoma cell line P-815 was grown in high glucose Dulbecco's Modified Eagle's Medium (Gibco) with 10% heat-inactivated fetal calf serum (ABI) and penicillin-streptomycin, 100 U/ml and 100 μg/ml, respectively (Gibco). Cells were plated at a density of 2.5×10^5/ml in a total of 8 ml in 25 cm^2 flasks. In experiments with MDMA, the cells were incubated with MDMA

overnight (18 h) and harvested the following day. The contents of each flask were pelleted by centrifugation and frozen at −80 °C until assayed for TPH activity.

2.5. Stereotaxic injections

Rats were maintained under methoxyflurane (metophane) anesthesia for all stereotaxic procedures. Animals were positioned in a Kopf model 900 small animal stereotaxic apparatus, the skull was exposed and a small burr hole was made above the site of the injection. The coordinates for all the injection sites were verified by dye injection on test animals prior to the administration of MDMA. For i.c.v. injections the coordinates used were: 1.0, 0.9-1.1, and 3.5 mm below the dura. The values for the raphe and substantia nigra injections were −7.5, 0, 6.0 and −5.3, 2.0, 7.5 mm, respectively. All injections were made relative to bregma according to Paxinos and Watson (1982). The volume for the i.c.v. injections was 20 μl while 2 μl volumes were used for both the nigral and raphe injections. MDMA was dissolved in saline while control animals receive saline alone. The latter injections were carried out over a 10-min period. After the injection the burr hole was sealed with bone wax and the scalp closed with wound clips. The animals were placed into individual cages for observation and allowed to recover for 3 h. Tissues were collected and stored as already described.

2.6. I.c.v. infusions

Animals were prepared for stereotaxic implantation as described above. All cannula components were purchased from Plastic Products Co. (Roanoke, VA). The placement of the cannulas was first verified by dye injection on test animals prior to implantation of an experimental group. Guide cannulas (22 gauge) were positioned in the right cerebral ventricle and attached with dental cement to a small machine screw also inserted in the skull. A dummy cannula was then inserted into the guide cannula, the scalp was closed with wound clips and the animals were allowed 2-3 days to recover. For the infusions, an internal cannula was inserted into the guide cannula and the animals were attached by a swiveled tether system (Stoelting Co., Chicago, IL) to a Razel Model A99 syringe pump (Stanford, CT). The animals were then placed in drum-shaped, clear plastic observation chambers for the duration of the experiment. One control (saline) and one MDMA animal were routinely infused simultaneously to allow a comparison of behavior. A constant infusion rate of 40 μl/h was used in all experiments to minimize changes in cerebrospinal fluid pressure as described by Osborne et al. (1986). At the termination of the experiment, the animals were disconnected from the infusion apparatus and sacrificed by decapitation. Samples of the brain regions were collected and stored as described above.

3. Results

3.1. Time course of the acute neurochemical effects of MDMA

Rats were administered 10 mg/kg MDMA by s.c. injection and sacrificed at various times thereafter for the determination of regional TPH activity as well as concentrations of 5-HT and 5-HIAA. Control animals received saline and were sacrificed at 3 h. MDMA produced similar changes in the measured parameters for all three regions. As shown in fig. 1, the loss of TPH activity uniformly preceded the decline in 5-HT concentrations. In the cortex, TPH activity was depressed as soon as 15 min after MDMA administration, while 5-HT levels were not significantly reduced until 30 min. Striatal TPH was similarly depressed by 15 min while the transmitter concentrations were not reduced to a significant extent until 2 h. In the hippocampus, TPH activity was significantly reduced by 30 min and 5-HT levels by 60 min. The concentration of the 5-HT metabolite, 5-HIAA increased in all three regions shortly after drug administration before paralleling the loss of 5-HT at 2-3 h. The rise in 5-HIAA was particularly prominent in the cortex and hippocampus. Similar results were observed in two separate experiments

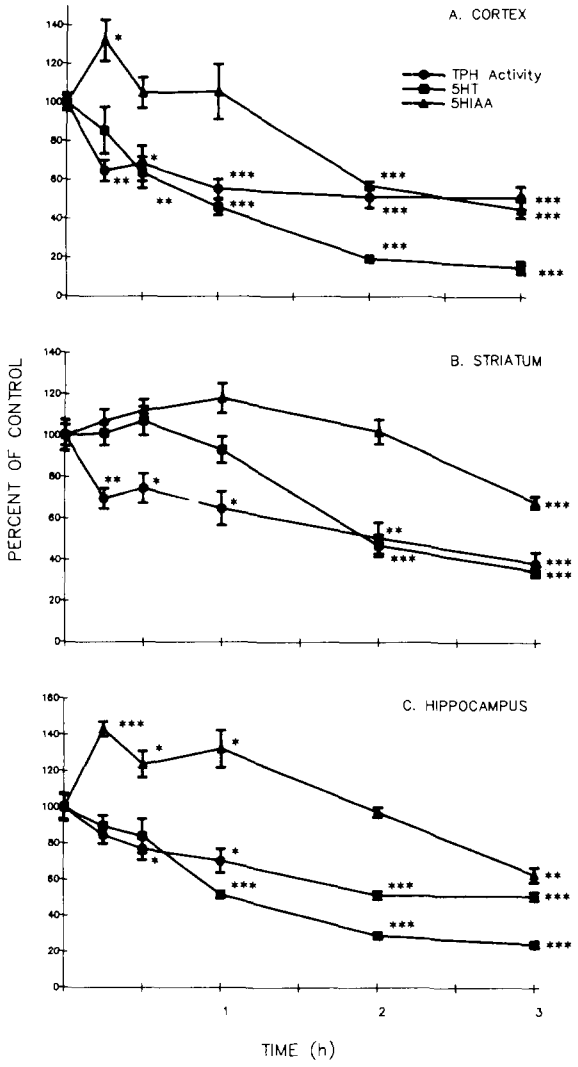

Fig. 1. Acute time course of the neurochemical changes following the administration of MDMA (10 mg/kg s.c.). Absolute control values for TPH activity (nmol oxidized/g per h) as well as 5-HT and 5-HIAA (μg/g) concentrations were, respectively: (A) 38.7 ± 1.8, 0.25 ± 0.01 and 0.14 ± 0.01; (B) 28.0 ± 2.0, 0.38 ± 0.03 and 0.38 ± 0.02; (C) 45.4 ± 3.5, 0.41 ± 0.03 and 0.29 ± 0.02. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. saline control by the two-tailed Student's t-test.

although the onset of the depression of TPH activity varied from 15 to 30 min.

The effect of MDMA on brainstem TPH activity was of a lesser magnitude than in the forebrain regions reaching significance only at the 3 h time point. In contrast to the 50-60% losses of enzyme activity in the serotonergic terminals of the hippocampus, cortex and striatum, TPH activity was reduced by only 25% in the brainstem (data not shown). Striatal concentrations of DA and its metabolites were also determined. DA concentrations were significantly elevated by MDMA to 134% of control by 60 min. DOPAC concentrations were reduced to 63% of control at the same time point. The concentration of HVA did not change significantly over the 3 h time course (data not shown).

3.2. Stereochemical requirements for the loss of TPH activity following MDMA administration

Rats were administered either the (+) or (−) stereoisomer of MDMA at a dose of 10 or 20 mg/kg and sacrificed 3 h later to determine if the loss of TPH activity following MDMA could be attributed to one enantiomeric form of the drug. The results shown in fig. 2 for cortical TPH activity indicate that either stereoisomer of MDMA can produce a depression of enzyme activity similar to that observed following administration of the racemate.

3.3. Effects of MDMA on serotonergic parameters in in vitro systems

In an effort to clarify the effect of MDMA on TPH activity, the effect of the drug on several in vitro models of serotonergic function was examined. One series of experiments utilized cortical slices continuously superfused with 250 μM

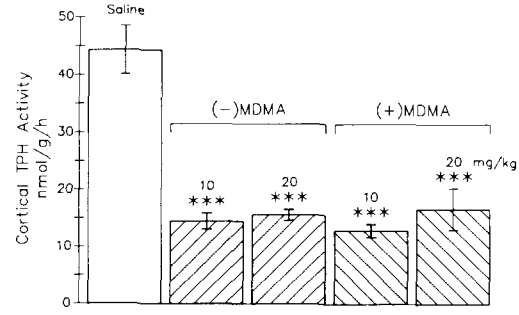

Fig. 2. Reduction in cortical TPH activity 3 h after the administration of (−) or (+) MDMA by s.c. injection. *** $P < 0.001$ vs. saline control by the two-tailed Student's t-test.

TABLE 1

Effect of MDMA on serotonergic parameters in cortical synaptosomes and superfused cortical slices.

	TPH nmol oxidized/mg protein per h
Superfused control	0.322 ± 0.022 (100 ± 6.8)
2.5×10^{-4} M MDMA	0.349 ± 0.043 (108.4 ± 13.4)
Frozen control	0.448 ± 0.055 (4)

MDMA in a KRB buffer containing glucose and constantly bubbled with 95% O_2-5% CO_2. In addition, 10 μM tryptophan was added to the KRB to maintain substrate levels for TPH. As shown in table 1, comparison with cortical slices kept frozen during the 2 h incubation indicated a small, but nonsignificant, loss of TPH activity during superfusion. However, exposure to MDMA had no additional effect on TPH activity in the slices. Similar results were observed with P_2 cortical synaptosomes incubated for 2 h at 37°C with concentrations of MDMA up to 100 μM. Although 5-HT and 5-HIAA concentrations decreased in the synaptosomes, TPH activity was unaffected by MDMA (data not shown).

In a final attempt to reproduce the effect of MDMA in vitro, we selected the murine mast cell line, P815, as an appropriate cell culture system to test. These cells contain easily measured levels of TPH activity as well as 5-HT (Levine et al., 1964). For these experiments, P815 cultures were incubated with or without 250 μM MDMA for 18 h. Subsequent measurement of TPH activity in the cultures showed MDMA to be without effect on enzyme activity (data not shown).

3.4. Effect of direct injections of MDMA into the rat brain

To determine if the acute effects of MDMA could be duplicated by direct application of the drug into the brain, three groups of rats were administered MDMA by stereotaxic injection under methoxyflurane anesthesia. The regions chosen as sites of injections were the cerebral ventricles, the dorsal raphe and the substantia nigra. Each animal was sacrificed 3 h after the injection and observed for behavioral effects during the intervening period. Under the conditions used, 300 μg MDMA was the highest dose tolerated since higher doses appeared to produce respiratory depression. In the case of the i.c.v. injections, neither cortical nor striatal TPH activity (not shown) were altered by MDMA. Cortical 5-HT levels on the contralateral side and hippocampal 5-HT concentrations were also unaffected by this treatment (not shown). A similar lack of effect on cortical TPH activity was observed when MDMA was injected directly into the area of the brain containing the anterior raphe nuclei. Striatal and hippocampal indole levels in the same animals were also unaffected by MDMA. There were no behavioral effects apparent with either the i.c.v. or brainstem injections.

The final set of injections were made into the right substantia nigra with striatal concentrations of dopamine, 5-HT and their metabolites being

TABLE 2

Effect of intranigral MDMA (300 μg) on ipsalateral and contralateral monoamine and metabolite concentrations.

	Dopamine (μg/g)	DOPAC (μg/g)	HVA (μg/g)	5-HT (μg/g)	5-HIAA (μg/g)
Saline					
Contralateral	11.3 ± 0.9	1.14 ± 0.20	0.82 ± 0.12	0.63 ± 0.04	0.58 ± 0.04
Ipsalateral	9.8 ± 0.4	1.74 ± 0.38	1.18 ± 0.25	0.60 ± 0.07	0.62 ± 0.06
I/C	(0.87)	(1.53)	(1.44)	(0.95)	(1.07)
MDMA					
Contralateral	9.2 ± 0.2	0.94 ± 0.03	0.66 ± 0.04	0.49 ± 0.04	0.63 ± 0.10
Ipsalateral	15.0 ± 1.3	3.80 ± 0.30	2.71 ± 0.22	0.57 ± 0.03	0.56 ± 0.03
I/C	(1.63)	(4.04)	(4.11)	(1.16)	(0.89)

determined in both the ipsilateral and contralateral striata at 3 h. Table 2 provides the right to left differences for both saline- and MDMA-injected animals. Saline-injected animals showed no significant differences in any of the measured parameters between the left and right striata; however, intranigral MDMA elevated dopamine and its metabolites in the striatum ipsilateral to the injection while having no effect on 5-HT or its metabolite, 5-HIAA. All animals in this group showed marked turning contralateral to the side of the injection.

3.5. Effect of i.c.v. infusions of MDMA on TPH activity and 5-HT concentrations

The lack of behavioral effects of intracranial injections of MDMA suggested that adequate brain levels of the drug may not be achieved or sustained following a single administration. Therefore, a constant i.c.v. infusion was used to insure that behaviorally relevant concentrations of MDMA could be maintained in the brain for a time period similar to that required to produce the neurochemical effects of MDMA after peripheral administration. Rats (approx. 270 g) were fitted with stainless steel cannulas in the right ventricle and allowed 2-3 days to recover from the surgery. In preliminary experiments using a 2 h infusion, a high dose of MDMA (6.5 mg/kg) was administered to insure a neurochemical effect. All MDMA-treated rats showed marked contralateral turning starting at approximately 1 h into the infusion periods. Cortical TPH activity in these animals was reduced to 67 ± 5% of control on the side of the infusion and 60 ± 6% on the contralateral side; hippocampal TPH activity was reduced by 30-40% on both sides.

The next experiment utilized a total dose of 2 mg/kg. Brain concentrations of MDMA were maintained by infusing the drug at a rate of 0.6 mg/h over 1 h then killing 2 h after the termination of the infusion. As is shown in fig. 3A, the direct cerebral infusion of MDMA produced a decrease in cortical and hippocampal TPH activity on the ipsilateral side of 33 and 36%, respectively ($P < 0.01$). Although striatal TPH activity appeared to be affected to a similar degree, the

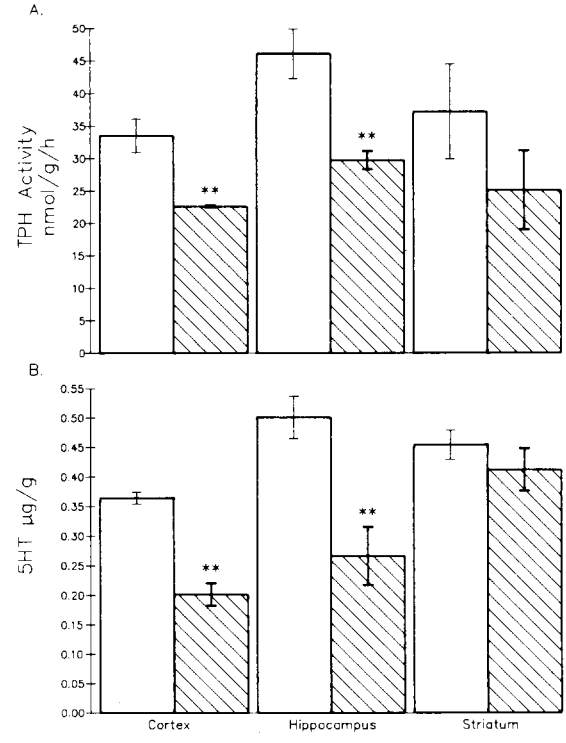

Fig. 3. Effect of continuous i.c.v. infusion of MDMA for 1 h at approximately 2 mg/kg cumulative dose on regional TPH activity (A) and 5-HT concentrations (B). Rats were sacrificed 2 h after the infusion ceased. N was 4 for each group. **P < 0.01 vs. saline control by the two-tailed Student's t-test. □ Saline; ▨ MDMA.

results were not statistically significant. The effect of MDMA on contralateral cortical and hippocampal 5-HT concentrations was similar to that observed for TPH activity with cortical levels of the transmitter decreasing 45% and hippocampal levels declining 47% (fig. 3B). Contralateral striatal 5-HT levels were not significantly altered by MDMA.

To insure that the effects observed with the infusion of the drug were due to a direct central effect of MDMA and could not have occurred after cycling through the periphery, an acute dose-response experiment was conducted in which rats were killed 3 h after the s.c. administration of 1, 2 or 4 mg/kg MDMA. As shown in fig. 4A, 1 mg/kg MDMA had no effect on cortical, hippocampal or striatal 5-HT concentrations; however, a small, but significant effect on the cortical levels

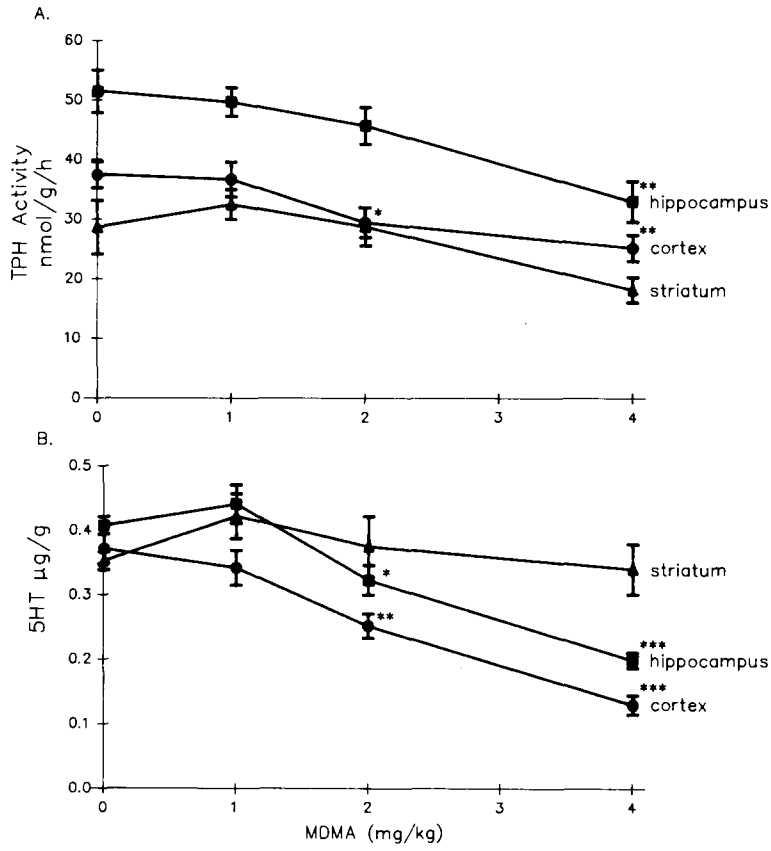

Fig. 4. Dose-response curves for the effect of peripherally administered MDMA on regional TPH activity (A) and 5-HT concentrations (B). MDMA was administered s.c. 3 h before sacrifice. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. saline control by the two-tailed Student's t-test.

was observed at 2 mg/kg. Only striatal 5-HT concentrations remained unaffected at 4 mg/kg MDMA. Changes in regional TPH activity exactly paralleled changes in transmitter concentrations (fig. 4B). Striatal enzyme activity was unaffected at any of the three doses used while cortical enzyme activity was depressed by 22 and 33% at 2 and 4 mg/kg, respectively. Hippocampal TPH activity was reduced only at the high dose of MDMA (36%).

The slight decrease in cortical TPH activity and 5-HT, after 2 mg/kg given by the peripheral route, left some question as to the possibility of such a dose having a peripheral effect even with central administration. To verify that the changes measured after centrally administered MDMA were due to a local effect, the infusion experiments were again repeated at a lower dose. In this case, slightly smaller animals (approximately 220 g) were infused for 1 h with a total dose of 1 mg/kg with the same paradigm as described above. The results of this experiment are shown in fig. 5. A pattern of changes in TPH activity very similar to that observed at the higher dose is apparent. Cortical and hippocampal enzyme activity fell approximately 31 and 39%, respectively, while there was no significant change in striatal TPH activity. When 5-HT concentrations in the contralateral tissues were analyzed it was found that neurotransmitter levels were not affected by MDMA in any of the three regions examined. Behaviorally, the MDMA and saline infused animals were almost indistinguishable with the only difference being that saline-treated rats generally slept dur-

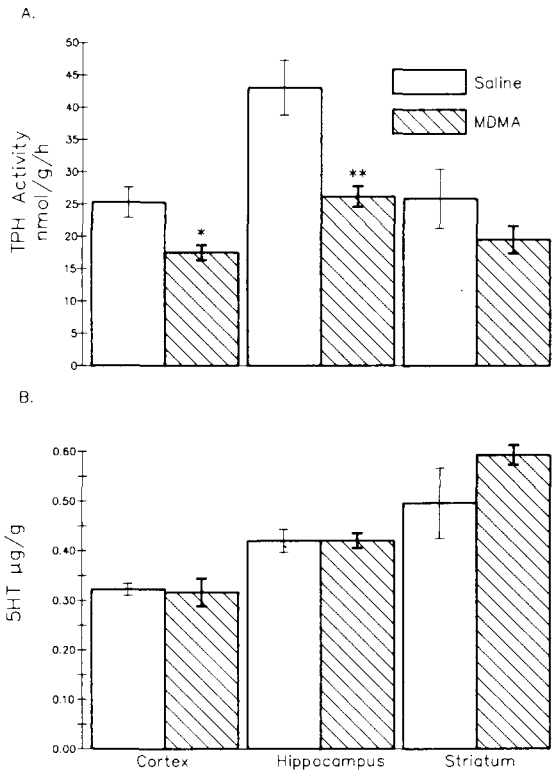

Fig. 5. Effect of continuous i.c.v. infusion of MDMA for 1 h at approximately 1 mg/kg cumulative dose on regional TPH activity (A) and 5-HT concentrations (B). Rats were sacrificed 2 h after the infusion ceased. N was 4 for each group. * $P < 0.05$; ** $P < 0.01$ vs. saline control by the two-tailed Student's t-test.

ing the experiment while MDMA-treated rats remained awake.

4. Discussion

The purpose of this study was to determine the requirements for the acute neurochemical effects of the psychedelic amphetamine analog, MDMA on serotonin synthesis. Previous work has demonstrated this effect is not merely an early phase of the neurotoxic action of the drug, but instead involves a loss in the V_{max} activity of TPH, and the rapid carrier-mediated release of 5-HT from the serotonergic neuron (Schmidt and Taylor, in press). Several observations support the claim that this acute effect is independent of the neurotoxicity including the demonstration that acute effect of MDMA can also be produced by its N-desmethyl and N-ethyl analogs, although the latter apparently does not produce neurotoxicity Schmidt, 1987b; Stone et al., 1987). These data and related experiments have shown that the acute effect of MDMA on TPH activity is a reversible phenomenon. In addition, while the neurotoxic effect of MDMA is a property of the (+) enantiomer of the drug, the acute depletion of 5-HT (Schmidt, 1987a) and the loss of TPH activity (fig. 2) occur with either stereoisomer.

We have previously demonstrated that the effect of MDMA on TPH activity is not a direct effect of the drug on the protein as it does not occur in homogenates (Schmidt and Taylor, in press). Attempts to interfere pharmacologically with the acute effect of MDMA have also met with limited success. Complete protection from MDMA-induced 5-HT depletion can be achieved with the simultaneous administration of 5-HT uptake inhibitors, although the loss of TPH activity is only partially blocked (Schmidt and Taylor, in press). Antagonists at 5-HT receptors also partially block the MDMA-induced loss of TPH activity possibly implicating serotonergic transmission in this effect (Schmidt and Taylor, in press).

The detailed time courses presented in this study show an increase in 5-HIAA that occurs prior to the loss of TPH activity which precedes the fall in 5-HT concentrations. Others have shown increases in 5-HIAA immediately after drugs altering TPH activity such as PCA and methamphetamine (Peat et al., 1985). This would also suggest an increase in 5-HT turnover is involved in the acute effect of MDMA on serotonergic neurons. Our results indicate that whatever action MDMA has on serotonergic neurons to trigger these neurochemical effects, the intact animal is required for its production. High concentrations of MDMA did not produce a significant loss of enzyme activity in either superfused cortical slices or cortical synaptosomes.

Taken together with the apparent lack of neurochemical effects after centrally injected MDMA, one plausible explanation for these results would be that the peripheral generation of an active metabolite is responsible for the acute neurochemical effect of MDMA. However, attempts to mod-

ify peripheral metabolism of MDMA with the enzyme inducer, phenobarbital, had no effect on MDMA-induced changes in TPH activity or 5-HT concentrations (Schmidt and Taylor, unreported observation). It might also be expected that the generation of an active metabolite of MDMA would be a stereoselective process as is the metabolism of PCA (Ames and Frank, 1982). However, the ability of MDMA to affect TPH activity is not due exclusively to either enantiomer. The inability of direct central injections of MDMA to produce any neurochemical alterations in serotonergic parameters was at first confusing. However, the observation that there were no behavioral effects of such a massive dose of centrally administered MDMA suggested a very short brain half-life for the drug under these conditions. Intranigral injections produced the only behavioral or neurochemical changes although these were restricted to the dopaminergic system. Interestingly, these changes contrast with those seen after peripheral administration of MDMA. Similar to other amphetamines, peripheral MDMA causes a transient elevation in DA levels with a drop in DOPAC concentrations. Intranigral injection of MDMA produced a sharp elevation of DA and both its metabolites. Taken together with the turning observed in these animals this would suggest a large increase in DA release in the striatum. However, intranigral injection of 10 μg amphetamine has been shown to reduce DA metabolites (Zetterstrom et al., 1986) indicating that either there is a qualitative change in effects as one increases the dose of an amphetamine or that MDMA has fundamentally different effects upon local injection than does amphetamine.

This lack of behavioral effects with intracerebrally administered MDMA led us to question the duration of time that brain concentrations of MDMA may need to be maintained to produce the acute effects of the drug on serotonergic neurons. Using tritiated MDA, Marquardt et al. (1978) showed that 10% of a peripherally administered dose of the drug was present in the brain of rats within 30 min of injection. By 1 h this value was down to 4% and by 2 h to 2%. For a 3 mg dose of MDMA this would translate to an approximate brain concentration of 300 μg which would be maintained for the first hour after drug administration. Infusion of 600 μg MDMA for 1 h (approximately 2 mg/kg total body dose) gave very clear neurochemical effects similar to those seen with the peripheral administration of much higher doses of MDMA (compare fig. 3 and fig. 4). The continuous infusion of MDMA for 1 h at one half this dose still produced the decrease in TPH activity. It is worth noting that the total amount of MDMA administered to the low dose infusion group is the same as that administered by bolus injection centrally. In the latter case however, MDMA had no effect on either TPH activity or 5-HT concentrations. Interestingly, there was no change in 5-HT concentration in any of the three regions examined at the lowest infusion dose. Close examination of the time course for the effect of MDMA on TPH activity and 5-HT concentrations reveals that TPH activity does decline before 5-HT concentrations. Therefore such a divergence in enzyme activity and transmitter levels can also occur with peripheral administration of MDMA.

The results, therefore, show that the acute effect of MDMA on serotonin synthesis in the rat brain is a centrally mediated event. It is likely that the acute, reversible effect of related drugs, such as PCA, are initiated in a similar manner. The interpretation of the results cannot be extended to the neurotoxic effects of these drugs since long-term effects of these drugs may well be mediated by a peripheral metabolite. There are, in fact, data in the literature to suggest that a metabolite of PCA can be formed which binds to cell macromolecules, although this metabolite has not been identified or linked to the production of neurotoxicity (Silverman and Ho, 1979).

Although we have demonstrated that the acute effect of MDMA on TPH activity is centrally mediated event, the mechanism for this effect remains elusive. Earlier work has not demonstrated a role for the dopaminergic system in this effect (Schmidt and Taylor, in press) as had been previously shown for methamphetamine (Schmidt et al., 1985). Depletion of serotonin stores with reserpine likewise did not modify the acute effects of MDMA, although it must be kept in mind that such treatment would not completely block MDMA-induced 5-HT release as reserpine only

depletes vesicular stores. Based on the rapid increase in the brain levels of 5-HIAA, the behavioral effects of the drug (Schechter, 1986) and the in vitro release data (Nichols et al., 1982; Johnson et al., 1986; Schmidt et al., 1987), the initial and predominant effect of MDMA in the rat brain is the increase in serotonergic transmission. This is also the single event produced by MDMA that can be interfered with to modify the acute effects of the drug on TPH activity. Not only do 5-HT receptor antagonists interfere with the decrease in TPH activity but 5-HT uptake blockers, such as citalopram, also antagonize the effect. In vitro experiments have previously demonstrated that citalopram blocks MDMA-induced 5-HT release as would be expected for a carrier-mediated process (Schmidt et al., 1987). It is also interesting to note that of the three areas examined in this study, the two showing the largest increases in 5-HIAA, i.e. the hippocampus and the cortex, also show the largest drop in TPH activity (compare fig. 1 with figs. 4 and 5). It therefore seems that at least some component of this acute effect of MDMA on TPH activity is due to a maintained elevation of serotonin release with a subsequent synaptic event. Further work using inhibitors of serotonergic transmission will test this proposal.

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References

Ames, M.M. and S.K. Frank, 1982, Stereochemical aspects of para-chloroamphetamine metabolism, Biochem. Pharmacol. 31, 5.

Battaglia, G., S.Y. Yeh, E. O'Hearn, M.E. Molliver, M.J. Kuhar and E.B. DeSouza, 1987, 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites, J. Pharmacol. Exp. Ther. 242, 911.

Bradford, M.M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

Braun, U., A.T. Shulgin and G. Braun, 1980, Centrally active N-substituted analogs of 3,4-methylenedioxyphenylisopropylamine (3,4-methylenedioxyamphetamine), J. Pharm. Sci. 69, 192.

Commins, D.L., G. Vosmer, R.M. Virus, W.L. Woolverton, C.R. Schuster and L.S. Seiden, 1987, Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain. J. Pharmacol. Exp. Ther. 241, 338.

Fuller, R.W., 1985, Persistent effect of amphetamine, p-chloroamphetamine and related compounds on central dopamine and serotonin neurons in rodents, Psychopharmacol. Bull. 21, 528.

Fuller, R.W., K.W. Perry and B.B. Molloy, 1975, Reversible and irreversible phases of serotonin depletion by 4-chloroamphetamine, European J. Pharmacol. 33, 119.

Hotchkiss, A.J. and J.W. Gibb, 1980, Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain, J. Pharmacol. Exp. Ther. 214, 257.

Hotchkiss, A.J., M. Morgan and J.W. Gibb, 1979, The long-term effects of multiple doses of methamphetamine on neostriatal tryptophan hydroxylase, tyrosine hydroxylase, choline acetyltransferase, and glutamate decarboxylase activities, Life Sci. 25, 1373.

Johnson, M.P., A.J. Hoffman and D.E. Nichols, 1986, Effects of the enantiomers of MDA, MDMA and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices, European J. Pharmacol. 132, 269.

Levine, R.J., W. Lovenberg and A. Sjoerdsma, 1964, Hydroxylation of tryptophan and phenylalanine on neoplastic mast cells of the mouse, Biochem. Pharmacol. 13, 1283.

Lovenberg, W. and K. Engelman, 1971, Assay of serotonin related metabolites and enzymes, Methods Biochem. Anal. 19, 1.

Marquardt, G.M., V. DiStefano and L.L. Ling, 1978, Metabolism of β-3,4-methylenedioxyamphetamine in the rat, Biochem. Pharmacol. 27, 1503.

Nichols, D.E., D.H. Lloyd, A.J. Hoffman, M.B. Nichols and G.K.W. Yim, 1982, Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes, J. Med. Chem. 25, 530.

Osborne, P.G., D.A. Denton and R.S. Weisinger, 1986, Cerebrospinal fluid pressure of anesthetized rats during intracerebroventricular infusion, Physiol. Behav. 37, 1.

Paxinos, G. and C. Watson, 1982, The Rat Brain in Stereotaxic Coordinates (Academic Press, New York).

Peat, M.A., P.F. Warren, C. Bakhit and J.W. Gibb, 1985, The acute effects of methamphetamine, amphetamine and p-chloroamphetamine on the cortical serotonergic system of the rat brain: evidence for differences in the effects of methamphetamine and amphetamine, European J. Pharmacol. 116, 11.

Ricaurte, G., G. Bryan, L. Strauss, L. Seiden and C. Schuster,

1985, Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals, Science 229, 986.

Ross, S.B., 1976, Antagonism of the acute and long-term biochemical effects of 4-chloroamphetamine on the 5-HT neurons in the rat brain by inhibitors of the 5-hydroxytryptamine uptake, Acta Pharmacol. Toxicol. 39, 456.

Sanders-Bush, E. and L.R. Steranka, 1978, Immediate and long-term effects of p-chloroamphetamine on brain amines, in: Serotonergic Neurotoxins, eds. J.H. Jakoby and L.D. Lytle (Ann. N.Y. Acad. Sci., New York) p. 208.

Schechter, M., 1986, Discriminative profile of MDMA, Pharmacol. Biochem. Behav. 24, 1533.

Schmidt, C.J., 1987a, Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, J. Pharmacol. Exp. Ther. 240, 1.

Schmidt, C.J., 1987b, Acute administration of methylenedioxymethamphetamine: comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogs, European J. Pharmacol. 136, 81.

Schmidt, C.J. and J.W. Gibb, 1985, Role of the dopamine uptake carrier in the neurochemical response to methamphetamine: effects of amfonelic acid, European J. Pharmacol. 109, 73.

Schmidt, C.J., J.A. Levin and W. Lovenberg, 1987, In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain, Biochem. Pharmacol. 36, 747.

Schmidt, C.J., J.K. Ritter, P.K. Sonsalla, G.R. Hanson and J.W. Gibb, 1985, Role of dopamine in the neurotoxic effects of methamphetamine, J. Pharmacol. Exp. Ther. 233, 539.

Schmidt, C.J. and V.L. Taylor, Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine, Biochem. Pharmacol. (in press).

Schmidt, C.J., L. Wu and W. Lovenberg, 1986, Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue, European J. Pharmacol. 124, 175.

Silverman, P.B. and B.T. Ho, 1979, p-Chloroamphetamine: evaluation of a brain metabolite, Commun. Psychopharmacol. 3, 291.

Stone, D.M., D.C. Stahl, G.R. Hanson and J.W. Gibb, 1986, The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain, European J. Pharmacol. 128, 41.

Stone, D.M., M. Johnson, G.R. Hanson and J.W. Gibb, 1987, A comparison of the neurotoxic potential of methylenedioxyamphetamine (MDA) and its methylated and N-ethylated derivatives, European J. Pharmacol. 134, 245.

Zellerström, T., T. Sharp and U. Ungerstedt, 1986, Further evaluation of the mechanism by which amphetamine reduces striatal dopamine metabolism: a brain dialysis study, European J. Pharmacol. 132, 1.