0022-3565/93/2652-0813$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 265, No. 2
Printed in U.S.A.
Copyright © 1993 by The American Society for Pharmacology and Experimental Therapeutics

Short-Term Effects of 2,4,5-Trihydroxyamphetamine, 2,4,5-Trihydroxymethamphetamine and 3,4-Dihydroxymethamphetamine on Central Tryptophan Hydroxylase Activity[1]

IKRAM ELAYAN, JAMES W. GIBB, GLEN R. HANSON, HENG KEANG LIM, ROGER L. FOLTZ and MICHEL JOHNSON

Department of Pharmacology and Toxicology (I.E., J.W.G., G.R.H., M.J.) and Center for Human Toxicology (H.R.I., R.L.F.), University of Utah, Salt Lake City, Utah

Accepted for publication January 21, 1993

ABSTRACT

In previous studies, we have reported the long-term effects of several metabolites of 3,4-methylenedioxymethamphetamine (MDMA) on tryptophan hydroxylase (TPH) activity. In this study, the short-term effects of three metabolites of MDMA, 2,4,5-trihydroxyamphetamine (THA), 2,4,5-trihydroxymethamphetamine (THM) and 3,4-dihydroxymethamphetamine, and the *in vitro* effect of THA on TPH activity are reported. After short-term treatment, hippocampal TPH activity was decreased to 8 and 54% of control in response to THA and THM, respectively, but was unaltered after 3,4-dihydroxymethamphetamine. Incubating TPH from THM-treated rats with dithiothreitol under nitrogen failed to reverse the decrease in enzyme activity induced by THM treatment. THA also decreased tyrosine hydroxylase activity to 75% of control, whereas the enzyme activity remained unaltered by THM. The structural analog of THA, 6-hydroxydopamine, failed to reproduce the effect of THA on TPH activity; however, 5,6-dihydroxytryptamine decreased hippocampal TPH activity to 18% of control. In the *in vitro* study, the hippocampus and the striatum were incubated in varying concentrations of THA. After a 1-h incubation at 37°C, hippocampal TPH activity was decreased to 83, 71, 68, 47 and 3% of control after exposure to 0.001, 0.01, 0.1, 0.5 or 5.0 mM THA, respectively; striatal TPH activity was reduced to 98, 95, 70, 54 and 17% of control, respectively. Incubating the enzyme under reducing conditions failed to restore the enzyme activity to control levels. These observations indicate that THA and THM produce unique *in vivo* and *in vitro* effects which are different from those of MDMA.

MDMA, a ring-substituted derivative of amphetamine, produces both long-term and short-term effects in the central serotonergic system. MDMA causes long-term alterations in the serotonergic parameters which include persisting marked reductions in levels of serotonin (5-HT) and its major metabolite, 5-HIAA, a decrease in the number of 5-HT reuptake sites and a decrease in TPH activity (Battaglia *et al.*, 1987, 1988; Schmidt *et al.*, 1986; Commins *et al.*, 1987; Stone *et al.*, 1986; O'Hearn *et al.*, 1988). These changes in serotonergic parameters reflect the destruction of serotonergic nerve terminals. TPH activity is decreased within minutes after the administration of MDMA (Stone *et al.*, 1987). In contrast to the persisting long-term effects of MDMA, the early short-term inactivation is completely reversed by prolonged anaerobic incubation of the enzyme in the presence of dithiothreitol and Fe^{++} (Stone *et al.*, 1989). This suggests that the inactivation of the enzyme produced shortly after treatment differs from that observed after several days.

Like MDMA, other amphetamine analogs such as methamphetamine (Peat *et al.*, 1985), fenfluramine (Steranka and Sanders-Bush, 1979), *p*-chloroamphetamine (Sanders-Bush *et al.*, 1972) and N-ethyl-3,4-methylenedioxyamphetamine (Johnson *et al.*, 1987a) induce a rapid inactivation of TPH activity which can be observed in frontal cortex, striatum and hippocampus. The inhibition of TPH activity induced by these amphetamine analogs is reversed by incubating the enzyme under reducing conditions in an anaerobic environment (Stone *et al.*, 1989; Johnson *et al.*, 1989). The mechanism by which MDMA and these amphetamine analogs produce this rapid decrease in TPH activity is still unknown. DA is suspected as a mediator because its depletion in the striatum prevented the

Received for publication September 22, 1992.
[1] This research was supported by USPHS grants DA 00869, DA 04222 (J.W.G., G.R.H., M.J.) and DA 05860 (R.L.F., H.K.L.).

ABBREVIATIONS: DA, dopamine; DHM, 3,4-dihydroxymethamphetamine; DHT, 5,6-dihydroxytryptamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HPLC-EC, high-performance liquid chromatography with electrochemical detection; 5-HT, 5-hydroxy-tryptamine; 5-HTP, 5-hydroxytryptophan; HVA, homovanillic acid; MDMA, 3,4-methylenedioxymethamphetamine; 6-OHDA, 6-hydroxydopamine; TH, tyrosine hydroxylase; THA, 2,4,5-trihydroxyamphetamine; THM, 2,4,5-trihydroxy-methamphetamine; TPH, tryptophan hydroxylase.

change induced by methamphetamine and MDMA (Johnson *et al.*, 1987b; Stone *et al.*, 1988); however, the role of this transmitter in other brain structures remains elusive. The formation of oxygen radicals and oxidative stress have been proposed as the cause of the rapid inactivation of TPH activity induced by MDMA (Stone *et al.*, 1989).

Seventeen metabolites of MDMA have been identified (Lim and Foltz, 1991a,b; Hirmatsu *et al.*, 1990). Many of these metabolites have been studied for their long-term effects on the central nervous system (Johnson *et al.*, 1992; Elayan *et al.*, 1992; Steele *et al.*, 1991; McCann and Ricaurte, 1991). Two of these metabolites, namely, THA and THM (fig. 1), produce long-term changes in the central serotonergic and dopaminergic systems that are distinct from those of MDMA (Johnson *et al.*, 1992; Elayan *et al.*, 1992; Zhao *et al.*, 1992). DHM is a metabolite that does not produce long-term effects on TPH activity (Johnson *et al.*, 1992).

The purpose of this study was to determine if the neurotoxic metabolites THA and THM and the nontoxic metabolite DHM produce short-term effects on TPH activity. Whether THA produces *in vitro* effects similar to those of MDMA was also determined.

Materials and Methods

In Vivo Study

Animal treatment. Male Sprague-Dawley rats, weighing 200 to 280 g, were housed two per cage in a temperature-controlled room (24°C) with a 12-h alternating light/dark cycle; all animals had access to food and water *ad libitum*.

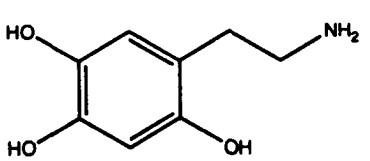
Experimental procedure. The rats were anesthetized with chloral hydrate (375 mg/kg, i.p.) and placed in a stereotaxic apparatus, and 1 μmol of THA, THM, DHM or DHT, or 1.2 μmol of 6-OHDA was injected into the right lateral ventricle (−0.8 mm from bregma, 1.4 mm lateral to the midline, 4.6 mm ventral to the surface of the skull according to Paxinos and Watson, 1982). All drugs were dissolved in 20 μl of 0.9% saline containing 0.1% sodium ascorbate and injected at a rate of 10 μl/min. The needle was left in place for 2 min after injection and the animals were sacrificed 3 h later. After decapitation, the hippocampus and the striatum were rapidly removed on a cold plate and kept frozen at −80°C until assayed.

In Vitro Study

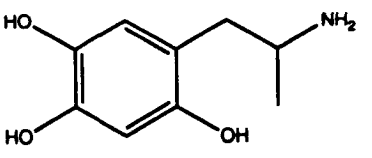
Experimental procedure. The hippocampus and striatum of male Sprague-Dawley rats were excised and incubated in 12×75 mm glass tubes containing 0.001, 0.01, 0.1, 0.5 or 5.0 mM THA dissolved in 1 ml of Ringer's buffer (118 mM NaCl, 4.84 mM KCl, 1.15 mM $MgSO_4$, 1.15 mM KH_2PO_4, 25 mM $NaHCO_3$, 11.1 mM glucose, 2.5 mM $CaCl_2$, pH 7.3). After a 1-h incubation at 37°C under a flow of 95% O_2 and 5% CO_2, the tissues were removed from the tubes, frozen on dry ice and stored at −80°C until they were assayed. The contralateral tissue served as control and was incubated in Ringer's buffer under the same conditions.

TPH assay. TPH activity was determined by measuring the formation of 5-HTP with a high performance liquid chromatography coupled with an electrochemical detector (HPLC-EC) as described elsewhere (Johnson *et al.*, 1992). Frozen tissues were weighed and homogenized in ice-cold 50 mM HEPES (Sigma Chemical Co., St. Louis, MO), pH 7.4, containing 0.2% Triton X-100, and 5 mM dithiothreitol (Calbiochem Corp., San Diego, CA) using a Potter-Elvehjem homogenizer. The striatum and the hippocampus were homogenized in 125 μl and 200 μl of the homogenizing buffer, respectively. The homogenates were centrifuged at 40,000 × *g* for 15 min at 4°C and duplicate 7.5-μl aliquots of the supernatant were assayed. Boiled supernatant was used for blanks. Five μl of a reaction mixture containing 240 nmol of HEPES, 10 mmol of tryptophan, 5.8 nmol of *m*-hydroxybenzylhydrazine (NSD 1015, Sigma Chemical Co.) and 17.5 nmol of *dl*-6-methyl-5,6,7,8-tetrahydropterin (Sigma Chemical Co.) were added to the enzyme extract. After a 30-min incubation at 37°C, the reaction was terminated by adding 100 μl of 0.2 N perchloric acid containing 32 ng of 5-HIAA as an internal standard. After centrifuging the tubes at 1,000 × *g* for 15 min, 5 μl of the supernatant were injected onto a 12.5-cm PartiSphere C_{18} reverse-phase column (Whatman Inc., Clifton, NJ) equipped with a 1-cm reverse-phase guard column or a 10-cm long 3-micron Microsorb C_{18} reverse-column (Rainin Instrument Co. Inc., Woburn, MA). The mobile phase consisted of 0.15 M monochloroacetic acid buffer (pH 2.9) containing 2 mM disodium EDTA, 0.1 mM 1-octanesulfonic acid sodium salt (Eastman Kodak Co., Rochester, NY) and 12.5% methanol. The electrochemical detector (model LC-4B; Bioanalytical Systems, Inc., West Lafayette, IN) equipped with a glassy carbon electrode was set at a potential of +0.6 V (*vs.* Ag/AgCl reference electrode). Peak heights of samples were quantified by comparing them with the peak heights of a known standard.

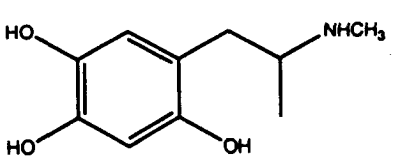
***In vitro* enzyme reactivation.** A 30-μl aliquot of the hippocampal supernatant with dithiothreitol (5 mM) was used for TPH assays and transferred into a 6×50 mm glass tube. The tubes were placed in a



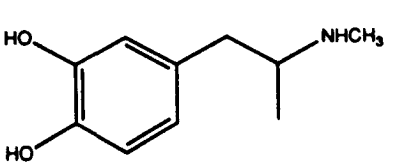
6-OHDA



THA



THM



DHM

Fig. 1. Chemical structure of THA, THM, DHM and 6-OHDA.

vacuum desiccator and incubated for 20 to 24 h under a slow flow of nitrogen gas at 25°C as previously described (Stone *et al.*, 1989). TPH activity was assayed immediately after the incubation.

TH assay. Striatal TH activity was determined by a modified method described by Nagatsu *et al.* (1983). Duplicate 10-μl aliquots from the supernatant were used for TPH activity and added to 40 μl of double-distilled water. After addition of 50 μl of a reaction mixture, each incubation medium contained 550,000 dpm of [3,5-^{3}H]tyrosine (54.2 Ci/mmol, New England Nuclear Research Products, Boston, MA), 10 nmol of *dl*-6-methyl-5,6,7,8-tetrahydrobiopterine, 10 nmol of 2-mercaptoethanol and 20 μmol of sodium acetate. The radiolabeled tyrosine was periodically purified by column chromatography using Dowex-50 resin (Sigma Chemical Co.) and stored in absolute ethanol at −20°C. The reaction medium was incubated for 15 min at 37°C, after which the reaction was stopped by the addition of 1 ml of a 7.5% (w/v) charcoal suspension in 1 N HCl as described by Reinhard *et al.* (1986). The mixture was centrifuged for 30 min at 2,000 × *g* and an aliquot of the supernatant was counted on a liquid scintillation detector (Packard, 2000 CA Tri-Carb, Downers Grove, IL).

Determination of neurotransmitter concentrations. The concentrations of 5-HT and its metabolite 5-HIAA, DA and its metabolites DOPAC and HVA were determined in the striatum contralateral to the drug injection site by HPLC-EC as described by Johnson *et al.* (1988).

Drug synthesis. A full description of the synthesis of THA, THM and DHM has been published elsewhere (Lim and Foltz, 1991a,b).

Statistical analysis. All data were analyzed by the unpaired Student's *t* test.

Results

In Vivo Studies

Effects of THA, THM and DHM on hippocampal and striatal TPH activity. Figure 2 depicts the effect of i.c.v. injection of the three MDMA metabolites on hippocampal and striatal TPH activity. The enzyme activity in the frontal cortex was not affected by THM treatment (data not shown). THA decreased hippocampal TPH activity to 8% of control, whereas striatal enzyme activity was decreased to 79% of control. A comparable response was seen with THM; hippocampal TPH activity was reduced to 54% of control, whereas striatal activity was reduced to 87% of control. The effect of DHM, however, differed from that of THM and THA in that DHM increased TPH activity in the striatum to about 128% of control and had no effect on hippocampal enzyme activity. Because DHM failed to mimic the acute effect of MDMA on TPH activity, the remainder of the study will focus on the effects of THA and THM.

Effects of THA and THM on striatal TH activity. Because THA and THM decreased TPH activity, we determined whether their effects were selective for the serotonergic system or whether they also affected the dopaminergic system. Administration of THA decreased TH activity to 75% of control, whereas THM produced no significant change in TH activity (fig. 3).

Effects of THA and THM on striatal DA, 5-HT and their metabolites. Although THA decreased TH activity (fig. 3), it actually increased DA levels to 135% of control in the contralateral striatum, which was accompanied by an increase in the levels of DOPAC and HVA to 287 and 237% of control, respectively (fig. 4A). THM failed to alter DA levels, but it increased DOPAC and HVA content to 512 and 366% of control, respectively (fig. 4B). THA and THM had different effects on 5-HT levels. Although THA increased the 5-HT level to 115% of control, THM decreased it to 74% of control. The

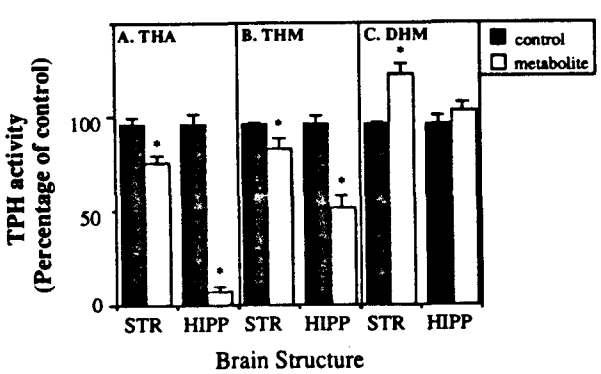


Fig. 2. Effects of A) THA, B) THM and C) DHM on striatal and hippocampal TPH activity. One μmol of THA, THM, DHM or the vehicle was administered by i.c.v. injection and rats were sacrificed 3 h later. The results are expressed as percentage of their respective controls (mean ± S.E.). The control values of TPH activity, expressed as nanomoles of hydroxylated tryptophan/h/g tissue, were: striatum (**STR**), 137 ± 6; hippocampus (**HIPP**), 75 ± 5 (n=5–8; * $P < .05$ *vs.* control).

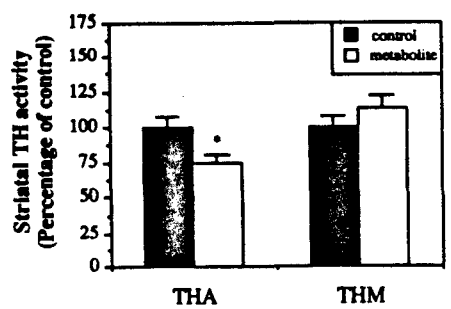


Fig. 3. Effects of THA and THM on striatal TH activity. The animals were treated as described in figure 2. The results are expressed as percentage of control (mean ± S.E.). The control value of TH activity was 1180 ± 92 nmol of hydroxylated tyrosine/h/g tissue (n=5–8; * $P < .05$ *vs.* control).

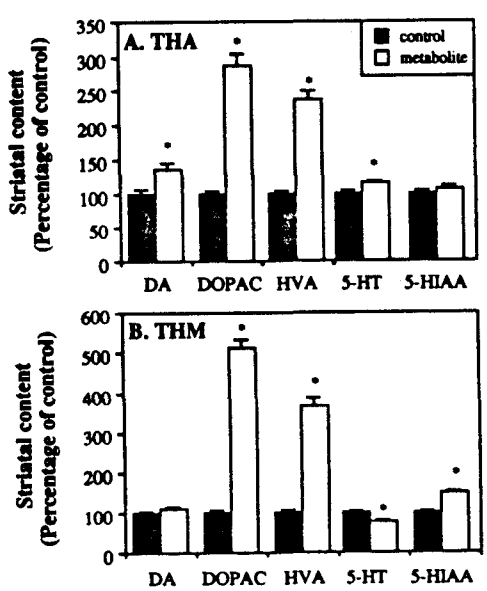


Fig. 4. Effects of A) THA and B) THM on striatal dopaminergic and serotonergic systems. The animals were treated as described in figure 2. The results are expressed as the percentage of control (means ± S.E.). The control values, expressed in μg/g tissue, were: DA, 9.0 ± 0.4; DOPAC, 0.77 ± 0.03; HVA, 0.91 ± 0.09; 5-HT, 0.67 ± 0.03; 5-HIAA, 0.66 ± 0.03 (n=5–8; * $P < .05$ *vs.* control).

content of 5-HIAA was not affected by THA treatment, but was increased to 149% of control with THM treatment.

Nature of the THM-induced decrease in hippocampal TPH activity. Because THM decreased hippocampal TPH activity (fig. 2), the reversibility of this effect was tested. Incubating the enzyme under reducing conditions failed to reverse the enzyme inhibition (fig. 5). TPH activity was 49% of control before reducing conditions and 55% of control after reducing conditions. We observed an increase in the enzyme activity after reducing conditions in both the control and treated groups when compared to the enzyme activity before incubation under reducing conditions (see fig. 5 legend). This increase in the enzyme activity is likely due to evaporation of the sample exposed to a flow of nitrogen (see "Materials and Methods"). This confounding change in the enzyme activity was reconciled by expressing the activity as percent of its own control (fig. 5).

Comparison with effects of 6-OHDA on TPH activity. Because THA and THM are structurally similar to 6-OHDA (fig. 1), it was of interest to determine the effect of 6-OHDA on TPH activity. As depicted in figure 6, 6-OHDA did not affect TPH activity in either the hippocampus or the striatum.

Comparison with effects of DHT on TPH activity. DHT is an established serotonergic neurotoxin. Lim and Foltz (1991b) reported that THA and THM cyclize, thus forming an indole molecule that is similar in structure to DHT. Because of this similarity, the effect of DHT on TPH activity was assessed. DHT decreased hippocampal TPH activity to 18% of control; incubating the enzyme under reducing conditions failed to significantly increase TPH activity (fig. 7).

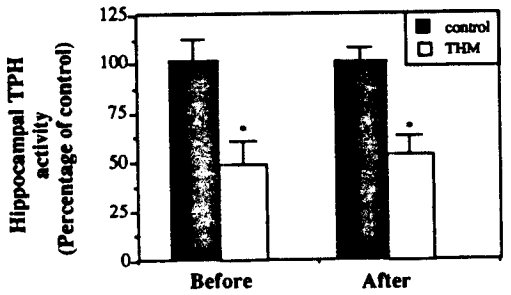


Fig. 5. The effects of THM on hippocampal TPH activity before and after reducing conditions (a 20–24 h anaerobic incubation in the presence of dithiothreitol). Animals were treated as described in figure 2. Results are expressed as percentage of control. The control values, expressed as nanomoles of hydroxylated tryptophan/h/g tissue, were 133.15 ± 14.5 before reducing conditions and 271.5 ± 20.4 after reducing conditions (means ± S.E.) (n=5–8; * $P < .05$ *vs.* control).

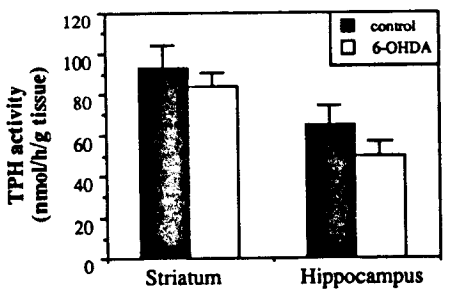


Fig. 6. Effects of 6-OHDA on hippocampal TPH activity. Animals received 1.2 μmol of 6-OHDA by i.c.v. injection and were sacrificed 3 h later. The results are expressed in nanomoles of hydroxylated tryptophan/h/g tissue (means ± S.E.) (n=5–8; * $P < .05$ *vs.* control).

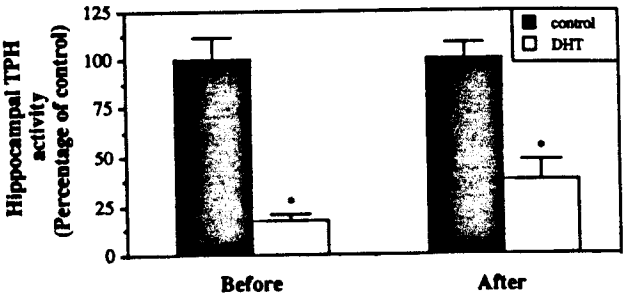


Fig. 7. Effect of DHT on hippocampal TPH activity before and after reducing conditions. Animals were treated as described in figure 2. Results are expressed as percentage of control. Control values, expressed as nanomoles of hydroxylated tryptophan/h/g tissue, were 133.5 ± 14.5 before reducing conditions and 271.5 ± 20.4 after reducing conditions (means ± S.E.) (n=5–8; * $P < .05$). TPH activity after reducing conditions was not significantly different from that before reducing conditions.

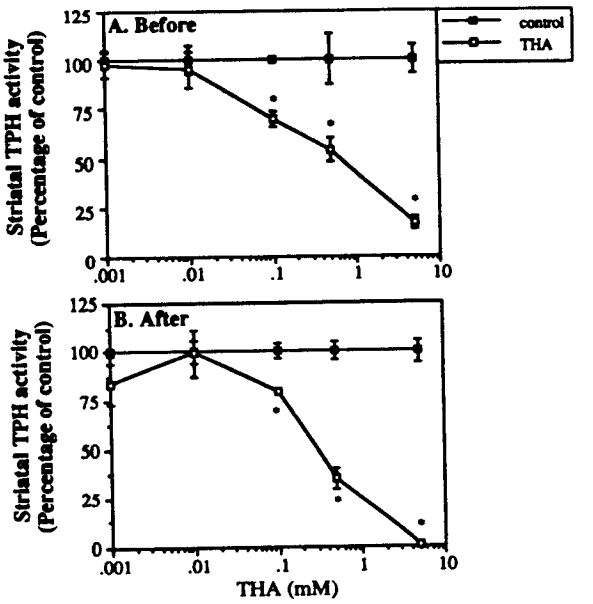


Fig. 8. *In vitro* effect of THA on striatal tryptophan hydroxylase activity before (A) and after reducing conditions (B). Striatum was excised and incubated with 0.001, 0.01, 0.1, 0.5 or 5.0 mM THA for 1 h under a flow of 95% O_2 and 5% CO_2. Reducing conditions are described in figure 5. Results are expressed as percentage of control (n=5–8; * $P < .05$ *vs.* control).

In Vitro Studies

***In vitro* effect of THA.** The *in vitro* effects of five different concentrations of THA (0.001, 0.01, 0.1, 0.5 and 5.0 mM) on striatal and hippocampal TPH activity are depicted in figures 8 and 9. In response to the three highest concentrations of THA (0.1, 0.5 and 5 mM), a decrease was observed in striatal TPH activity to 70, 54 and 17% of control, respectively (fig. 8A). Hippocampal TPH activity was decreased to 71, 68, 47 and 3% of control in response to 0.01, 0.1, 0.5 and 5.0 mM THA, respectively (fig. 9A). The hippocampus was more sensitive in that it also responded to the 0.01 mM concentration. Incubation of the enzyme under reducing conditions did not restore the enzyme activity to control levels (figs. 8B and 9B).

Discussion

In the present study, the short-term neurochemical effects of metabolites of MDMA, THA, THM and DHM were inves-

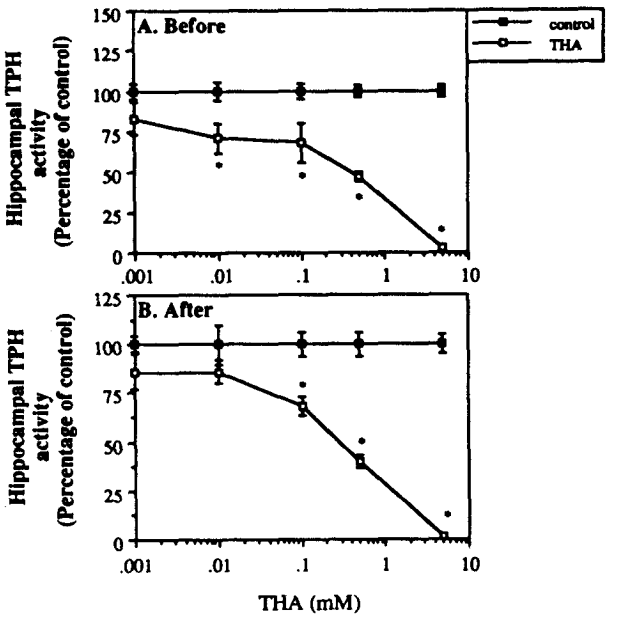


Fig. 9. *In vitro* effect of THA on hippocampal tryptophan hydroxylase activity before (A) and after reducing conditions (B). Hippocampus was excised and incubated with 0.001, 0.01, 0.1, 0.5 or 5.0 mM THA for 1 h under a flow of 95% O_2 and 5% CO_2. Reducing conditions are described in figure 5. Results are expressed as percentage of control (n=5–8; * $P < .05$ *vs.* control).

tigated. Both THA and THM, but not DHM, decreased TPH activity shortly after i.c.v. administration. The inactivation of TPH that occurs shortly after systemic administration of MDMA is reversed by incubating the enzyme under reducing conditions. In contrast, the inactivation of TPH activity by THA or THM was not reversed under reducing conditions. Incubating striatal and hippocampal tissues with THA *in vitro* produced a decrease in TPH activity similar to the decrease observed *in vivo*. This study demonstrated that THA or THM induces a rapid decrease in TPH activity which is distinct from the decrease induced by systemic administration of MDMA. Thus, it is unlikely that these metabolites of MDMA mediate their short-term effect on TPH activity by the same mechanism as does MDMA.

Stone *et al.* (1987, 1989) showed that within minutes after a single dose of MDMA, hippocampal TPH activity declines; incubating TPH under reducing conditions restores the enzyme activity. They proposed that oxidative stress is responsible for this rapid decrease in TPH activity, possibly by oxidation of a sulfhydryl group on TPH. Our data indicate that the inactivation of TPH by MDMA differs from that produced by its metabolites because the enzyme from rats treated with these metabolites were not reactivated when incubated under reducing conditions. Thus, the possibility that oxidation of a sulfhydryl group on TPH causes the inactivity produced by these metabolites is unlikely. There is a possibility that these metabolites form a quinone that binds to an essential sulfhydryl group in the enzyme, causing its inactivation. This possibility is supported by the observation that catecholamines, which are structurally similar to these metabolites, are oxidized to quinones (Marquardt and DiStefano, 1974) that can bind to a sulfhydryl group on albumin (Ito *et al.*, 1988). Interestingly, this type of condensation is not reversed by incubation under reducing conditions (Rotman *et al.*, 1976). Lim and Foltz have proposed a pathway by which THA and THM undergo cyclization through a quinone intermediate (Lim and Foltz, 1991b). Therefore, it is possible that the quinone molecule results in the inhibition of TPH by a similar condensation.

Because Lim and Foltz (1991b) demonstrated that THA and THM can undergo cyclization, resulting in the formation of an indole molecule quite similar to the structure of DHT, except for the absence of an aminoethyl side chain, we tested the possibility that this latter neurotoxin could mimic the effect of MDMA metabolites on TPH activity. Similar to THA and THM, DHT decreased TPH activity shortly after it was administered. It is possible that THA and THM produce their effects through cyclization to indole molecules; however, it is unlikely that this cyclized structure, which lacks the aminoethyl group, can be transported into the serotonergic neurons through the reuptake carrier. Thus, if the cyclized molecule is responsible for TPH inactivation, this cyclization would have to occur in the serotonergic nerve terminal. However, the finding that 6-OHDA did not affect TPH activity contradicts the possibility that the indole molecule mediates this short-term effect, because 6-OHDA can undergo cyclization to a molecule that is similar in structure to DHT and the cyclized form of THA and THM. The lack of an effect with 6-OHDA treatment was not due to its degradation by monoamine oxidase (MAO) because pargyline, a MAO inhibitor, given 1 h before 6-OHDA administration did not change the effects observed with 6-OHDA alone (results not shown). Thus, it appears unlikely that the ability of a molecule to cyclize to an indole-like structure is sufficient to explain this inactivation of TPH.

The observed increase in DA and its metabolites after THA treatment might be explained by an increase in the release of DA. It is noteworthy that the transmitters and their metabolites were measured in the brain structure contralateral to the side assayed for enzyme activity. After a unilateral 6-OHDA lesion, Robinson and Wishaw (1988) observed that DA release is increased in the contralateral side. Our results may reflect a similar phenomenon, because we also observed an increase in DA and its metabolites in the contralateral side. This increase might reflect a compensation for the decreased enzyme activity on the side of injection. Consistent with this proposal is our observation that the DA content was increased by THA, but not by THM. The increase in DOPAC and HVA by both drugs suggests, however, that both substances stimulate DA release. In the serotonergic system, we observed an increase in 5-HT only with THA, but not with THM. This might also be explained by the large inhibition of TPH activity in the contralateral side by THA in comparison to that produced by THM.

The finding that i.c.v. injection of THA decreased TPH activity prompted us to test if this effect can be reproduced *in vitro*. The effect of THA on striatal and hippocampal TPH activity *in vitro* was similar to that observed after i.c.v. injection. This observation indicates that the mechanism by which TPH activity is decreased by THA administration *in vitro* or by i.c.v. injection might be the same. By comparison, MDMA does not decrease TPH activity after i.c.v. injection nor as a result of *in vitro* incubation (Schmidt and Taylor, 1987, 1988). These findings demonstrate that MDMA decreases TPH activity in a manner distinct from that of THA. Incubating the enzyme treated with THA *in vitro* under reducing conditions failed to reverse the enzyme activity, which might indicate that the inhibition observed *in vitro* does not involve an oxidative process similar to that suggested for the short-term effect of MDMA.

In conclusion, two metabolites of MDMA, THA and THM, decrease TPH activity after short-term treatment. This effect is comparable to the short-term effect of systemically administered MDMA on enzyme activity. These metabolites produce effects, however, that are not congruous with those produced by MDMA. Thus, *in vivo* formation of these metabolites does not explain the mechanism by which MDMA produces its short-term effects on the central serotonergic system.

Acknowledgment

The authors wish to thank Mr. Keith Elkins for his technical assistance.

References

BATTAGLIA, G., YEH, S. Y. AND DE SOUZA, E. B.: MDMA-induced neurotoxicity: Parameters of degeneration and recovery of brain serotonin neurons. Pharmcol. Biochem. Behav. **29:** 269–274, 1988.

BATTAGLIA, G., YEH, S. Y., O'HEARN, E., MOLLIVER, M. E., KUHAR, M. J. AND DE SOUZA, E. B.: 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:** 911–916, 1987.

COMMINS, D. L., VOSMER, G., VIRUS, R. M., WOOLVERTON, W. L., SCHUSTER, C. R. AND SEIDEN, L. S.: Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain. J. Pharmacol. Exp. Ther. **241:** 338–345, 1987.

ELAYAN, I., GIBB, J. W., LIM, H. K., FOLTZ, R. L., HANSON, G. R. AND JOHNSON, M.: Long-term alteration in the central monoaminergic systems of the rat by 2,4,5-trihydroxyamphetamine but not by 2-hydroxy-4,5-methylenedioxymethamphetamine or 2-hydroxy-4,5-methylenedioxyamphetamine. Eur. J. Pharmacol. **221:** 281–288, 1992.

HIRAMATSU, M., KUMAGAI, Y., UNGER, S. E. AND CHO, A. K.: Metabolism of methylenedioxymethamphetamine: Formation of dihydroxymethamphetamine and a quinone identified as its glutathione adduct. J. Pharmacol. Exp. Ther. **254:** 521–527, 1990.

ITO, S., KATO, T. AND FUKITA, K.: Covalent binding of catechols to proteins through the sulfhydryl group. Biochem. Pharmacol. **37:** 1707–1710, 1988.

JOHNSON, M., ELAYAN, I., HANSON, G. R., FOLTZ, R. L., GIBB, J. W. AND LIM, H. K.: Effects of 3,4-dihydroxymethamphetamine and 2,4,5-trihydroxymethamphetamine, two metabolites of 3,4-methylenedioxymethamphetamine, on central serotonergic and dopaminergic systems. J. Pharmacol. Exp. Ther. **261:** 447–453, 1992.

JOHNSON, M., HANSON, G. R. AND GIBB, J. W.: Effects of N-ethyl-3,4-methylenedioxyamphetamine (MDE) on central serotonergic and dopaminergic systems of the rat. Biochem. Pharmacol. **36:** 4085–4093, 1987a.

JOHNSON, M., HANSON, G. R. AND GIBB, J. W.: Characterization of acute N-ethyl-3,4-methylenedioxyamphetamine (MDE) action on the central serotonergic system. Biochem. Pharmacol. **38:** 4333–4338, 1989.

JOHNSON, M., LETTER, A. A., MERCHANT, K., HANSON, G. R. AND GIBB, J. W.: Effect of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxyamphetamine isomers on central serotonergic and nigral neurotensin systems of the rat. J. Pharmacol. Exp. Ther. **244:** 977–982, 1988.

JOHNSON, M., STONE, D. M., HANSON, G. R. AND GIBB, J. W.: Role of the dopaminergic nigrostriatal pathway in methamphetamine-induced depression of the neostriatal serotonergic system. Eur. J. Pharmacol. 135: 231–234, 1987b.

LIM, H. K. AND FOLTZ, R. L.: In vivo formation of aromatic hydroxylated metabolites of 3,4-(methylenedioxy)methamphetamine in the rat: Identification by ion trap ms/ms and ms/ms/ms techniques. Biol. Mass Spectrom. **20:** 677–686, 1991a.

LIM, H. K. AND FOLTZ, R. L.: Ion trap tandem mass spectrometric evidence for the metabolism of 3,4-(methylenedioxy)methamphetamine to the potent neurotoxins 2,4,5-trihydroxymethamphetamine and 2,4,5-trihydroxyamphetamine. Chem. Res. Toxicol. **4:** 626–632, 1991b.

MARQUARDT, G. M. AND DISTEFANO, V.: The hepatic microsomal metabolism of beta-3,4-methylenedioxyamphetamine (MDA). Life Sci. **15:** 1603–1610, 1974.

MCCANN, U. D. AND RICAURTE, G. A.: Major metabolites of (±) 3,4-methylenedioxyamphetamine (MDA) do not mediate its toxic effects on the brain serotonin neurons. Brain Res. **545:** 279–282, 1991.

NAGATSU, T.: Analysis of monooxygenases in catecholamine biosynthesis: Tyrosine hydroxylase and dopamine-β-hydroxylase. *In* Methods in Biogenic Amine Research, ed. by S. Parvez, T. Nagatsu, I. Nagatsu and H. Parvez, pp. 329–357, Elsevier Science Publisher B. V., Amsterdam, 1983.

O'HEARN, E., BATTAGLIA, G., DE SOUZA, E. B., KUHAR, M. J. AND MOLLIVER, M. E.: Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause ablation of serotonin axon terminals in the forebrain: Immunocytochemical evidence of neurotoxicity. J. Neurosci. **8:** 2788–2800, 1988.

PAXINOS, G. AND WATSON, C.: The Rat Brain in Stereotaxic Coordinates, Academic Press, New York, 1982.

PEAT, M. A., WARREN, P. F., BAKHIT, C., AND GIBB, J. W.: 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. Eur. J. Pharmacol. **116:** 11–16, 1985.

REINHARD JR., J. F., SMITH, G. K. AND NICHOL, C. A.: A rapid and sensitive assay for tyrosine-3-monooxygenase based upon the release of ^3H_2O and adsorption of [^{3}H]-tyrosine by charcoal. Life Sci. **39:** 2185–2189, 1986.

ROBINSON, T. E. AND WISHAW, I. Q.: Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lession of the substantia nigra: a microdialysis study in freely moving rats. Brain Res. **450:** 209–224, 1988.

ROTMAN, A., DALY, J. W. AND CREVELING, C. R.: Oxygen dependent reaction of 6-hydroxydopamine, 5,6-dihydroxytryptamine and related compounds with proteins in vitro: s model for cytotoxicity. Mol. Pharmacol. **12:** 887–899, 1976.

SANDERS-BUSH, E., BUSHING, J. A. AND SULSER, F.: p-Chloroamphetamine-inhibition of cerebral tryptophan hydroxylase. Biochem. Pharmacol. **21:** 1501–1510, 1972.

SCHMIDT, C. J. AND TAYLOR, V. L.: Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem. Pharmacol. **36:** 4095–4102, 1987.

SCHMIDT, C. J. AND TAYLOR, V. L.: Direct central effects of acute methylenedioxymethamphetamine on serotonergic neurons. Eur. J. Pharmacol. **156:** 121–131, 1988.

SCHMIDT, C. J., WU, L. AND LOVENBERG, W.: Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue. Eur. J. Pharmcol. **124:** 175–178, 1986.

STEELE, T. D., BREWSTER, W. K., JOHNSON, M. P., NICHOLS, D. E. AND YIM, G. K. W.: Assessment of the role of α-methylepinine in the neurotoxicity of MDMA. Pharmacol. Biochem. Behav. **38:** 345–351, 1991.

STERANKA, L. R. AND SANDERS-BUSH, E.: Long-term effects of fenfluramine on the central serotonergic mechanisms. Neuropharmacology **18:** 895–903, 1979.

STONE, D. M., JOHNSON, M., HANSON, G. R. AND GIBB, J. W.: Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. **247:** 79–87, 1988.

STONE, D. M., JOHNSON, M., HANSON, G. R. AND GIBB, J. W.: Acute inactivation of tryptophan hydroxylase by amphetamine analogs involves the oxidation of sulfhydryl sites. Eur. J. Pharmacol. **172:** 93–97, 1989.

STONE, D. M., MERCHANT, K. M., HANSON, G. R. AND GIBB, J. W.: Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rats. Neuropharmacology **26:** 1677–1683, 1987.

STONE, D. M., STAHL, D. C., HANSON, G. R. AND GIBB, J. W.: The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic system in the rat brain. Eur. J. Pharmacol. **128:** 41–48, 1986.

ZHAO, Z., CASTAGNOLI JR., N., RICAURTE, G. A., STEEL, T. E. AND MARTELLO, M.: Synthesis and neurotoxicological evaluation of putative metabolites of serotonergic neurotoxin 2-(methylamino)-1-[3,4(methylenedioxy)phenylpropane[(*methylene dioxy) methamphetamine*]. Chem. Res. Toxicol. **5:** 89–94, 1992.

Send reprint requests to: Dr. James W. Gibb, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.