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Effects of 3,4-Dihydroxymethamphetamine and 2,4,5-Trihydroxymethamphetamine, Two Metabolites of 3,4-Methylenedioxymethamphetamine, on Central Serotonergic and Dopaminergic Systems

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ABSTRACT

The effects of 3,4-dihydroxymethamphetamine (DHM) and 2,4,5-trihydroxymethamphetamine (THM) on central serotonergic and dopaminergic systems were investigated to determine if these metabolites share the neurochemical properties of 3,4-methylenedioxymethamphetamine. THM (50–200 μg) or DHM (135 μg) was administered i.c.v. to rats; 5 days later, cortical, striatal and hippocampal tryptophan hydroxylase (TPH) activity were decreased by THM in a dose-dependent manner, whereas DHM was without effect in these brain structures. The concentration of serotonin in the brain structures contralateral to the side of THM injection was also decreased, but to a lesser degree. THM (100 and 200 μg) increased TPH activity to 155% of control in the dorsal raphe, whereas a dose of 50 μg increased TPH activity to 132% of control in the median raphe nucleus. THM also markedly reduced striatal tyrosine hydroxylase activity, but did not alter enzyme activity in the substantia nigra; DHM increased striatal tyrosine hydroxylase activity to 115% of control. These results suggest that THM, but not DHM, is toxic to both dopaminergic and serotonergic nerve terminals. Although THM could not be established as the neurotoxic metabolite explaining 3,4-methylenedioxymethamphetamine (MDMA) toxicity, its properties may prove useful in elucidating amphetamine toxicity.

MDMA destroys serotonergic nerve terminals, as reflected by a long-lasting decline in central TPH activity, a decrease in the concentration of 5-HT and 5-HIAA (Schmidt *et al.*, 1986; Schmidt, 1987; Stone *et al.*, 1987a,b) as well as by the decline in the number of 5-HT uptake sites (Battaglia *et al.*, 1987; Commins *et al.*, 1987; Schmidt, 1987). In contrast to several other neurotoxins, serotonergic cell bodies resist MDMA toxicity (O'Hearn *et al.*, 1988). The actual mechanism leading to the destruction of serotonergic nerve terminals is not well understood. It is unlikely that MDMA itself directly induces the toxic effect on neurons because direct injection of this amphetamine analog into the brain fails to induce neurotoxicity (Molliver *et al.*, 1986; Paris and Cunningham, 1990). As a result, there is considerable interest in the characterization of the neurochemical effects of the metabolites of MDMA.

The metabolism of MDMA has been extensively investigated in the rat by Lim and Foltz (Lim and Foltz, 1988, 1991a,b), who have demonstrated thus far 17 metabolites formed by N-demethylation, O-dealkylation, aromatic hydroxylation, deamination and conjugation. One of these metabolites, DHM, is oxidized to a quinone which was isolated as a glutathione adduct (Hiramatsu *et al.*, 1990). This together with the report of the formation of DHM in the brain (Lim and Foltz, 1988) suggest that this metabolite may contribute to the neurotoxicity induced by MDMA. Another metabolite may also participate in MDMA toxicity. THM is formed from the metabolism of 2-hydroxy-4,5-methylenedioxymethamphetamine (Lim and Foltz, 1988, 1990, 1991b; Lim et al., 1991b) and is expected to be neurotoxic due to its structural similarity to the potent catecholaminergic neurotoxin, 6-hydroxydopamine (fig. 1). Thus, the purpose of this study was to evaluate the effects of THM and DHM on the central serotonergic and dopaminergic systems in order to determine their possible role in MDMA-induced neurotoxicity.

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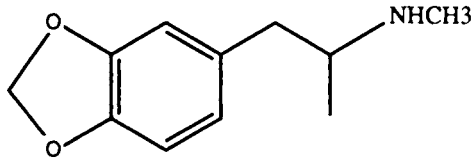
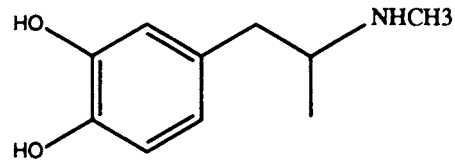
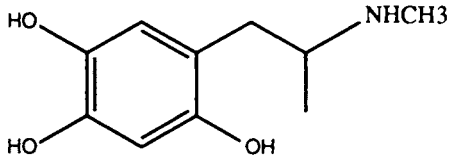
ABBREVIATIONS: DA, dopamine; DHM, 3,4-dihydroxymethamphetamine; FCx, frontal cortex; STR, striatum; HIP, hippocampus; SN, substantia nigra; DR, dorsal raphe; MR, median raphe; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC-EC, high-performance liquid chromatography with electrochemical detection; HVA, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; TH, tyrosine hydroxylase; THM, 2,4,5-trihydroxymethamphetamine; TPH, tryptophan hydroxylase; MDMA, 3,4-methylenedioxymethamphetamine.



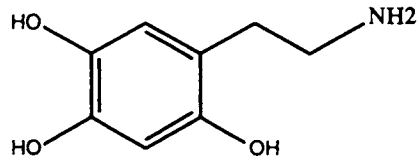
3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)



3,4-DIHYDROXYMETHAMPHETAMINE (DHM)



2,4, 5-TRIHYDROXYMETHAMPHETAMINE (THM)



6-HYDROXYDOPAMINE (6OHDA)

Fig. 1. Chemical structures of MDMA, DHM, THM and 6-hydroxydopamine.

Methods

Animals. Male Sprague-Dawley rats (Simonsen Laboratories Inc., Gilroy, CA) weighing 180 to 250 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*.

Drug synthesis. The synthesis of (±)DHM hydrobromide has been reported elsewhere (Lim and Foltz, 1988; Lim *et al.*, 1991a). (±)THM hydrobromide was synthesized from 2,4,5-trimethoxymethamphetamine, which was synthesized from 2,4,5-trimethoxybenzaldehyde by modification of the procedure for 4-hydroxy-3-methoxymethamphetamine (Lim and Foltz, 1988). The resultant crude 2,4,5-trimethoxymethamphetamine was purified by silica gel (60–120 mesh) column chromatography and eluted initially with toluene (125 ml), followed by chloroform (125 ml) and finally with toluene/chloroform/methanol (2:2:1). Evaporation of the pooled fractions gave a yellow-brown oil: GC/CI-MS [trifluoroacetyl derivative], m/z 336 [MH^+, base peak. After demethylation of the 2,4,5-trimethoxymethamphetamine by boron tribromide and subsequent quenching of the reaction with methanol (Lim and Foltz, 1988), the solvent was removed using a rotary evaporator before lyophilization. The resulting residue was identified as THM by high-resolution mass spectrometry and [^{1}H]NMR: m/z 197.1065 (M^+) (calculated for $C_{10}H_{15}NO_3$, 197.1052) [^{1}H]nuclear magnetic resonance (^2H_2O), δ 1.20 (d, J = 6.7 Hz, 3H, CCH_3), 2.63 (s, 3H, NCH_3), 2.77 (m, 2H, CH_2), 3.43 (m, 1H, CH), 6.44, 6.66 (2s, 2H, aromatic).

Experimental procedures. The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), placed in a stereotaxic apparatus and injected i.c.v. −0.8 mm from the bregma, 1.4 mm lateral to the midline and 4.6 mm ventral to the surface of the skull according to Paxinos and Watson (1982) with THM or DHM dissolved in 0.9% saline containing 0.1% sodium ascorbate. A 20-μl injection was made at a rate of 10 μl/min, after which the needle remained in place for an additional 5 min before being withdrawn. The animals were sacrificed by decapitation 5 days later, and the FCx (corresponding to the pregenual part of the anteromedial cortex as described by Emson and Lindvall, 1979), STR and HIP were rapidly removed on a cold plate; all tissues were stored at −80°C until assayed. The SN and the DR and MR were dissected out from frozen brain slices with a microdissecting knife and a punch.

Determination of TPH activity. TPH activity was determined with HPLC-EC by measuring the formation of 5-HTP resulting from the hydroxylation of tryptophan according to a modification of the methods described by Boadle-Biber *et al.* (1986), Friedman *et al.* (1972) and Hotchkiss *et al.* (1979). Frozen tissues ipsilateral to the side of THM or DHM injection were weighed and homogenized in 50 mM HEPES buffer (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-Elvejhem homogenizer. The following volumes, expressed in microliters, were used for these tissues: FCx, 100; STR, 125; HIP, 200; DR, 100; MR, 100. The protein content in the DR and MR homogenates was determined using the method of Bradford (1976). The homogenates were centrifuged at 40,000 × *g* for 15 min at 4°C and duplicate 7.5-μl aliquots of the supernatant were transferred into 6 × 50-mm glass tubes. Boiled supernatant was used for blanks. Five μl of a reagent mixture containing 240 nmol of HEPES, 10 nmol of tryptophan, 5.8 nmol of *m*-hydroxybenzylhydrazine (Sigma) and 17.5 nmol of *dl*-6-methyl-5,6,7,8-tetrahydropterin (Sigma) were added to the enzyme extract. The tubes were incubated at 37°C for 30 min and the reaction was stopped by transferring the tubes into an ice water bath followed by the addition of 100 μl of 0.2 N perchloric acid containing 32 ng of 5-HIAA (used as internal standard). The tubes were centrifuged at 1,000 × *g* for 15 min at 4°C; 5 μl of supernatant were injected onto a 12.5-cm PartiSphere C18 reverse-phase column (Whatman Inc., Clifton, NJ) equipped with a 1-cm R.P. guard column, or a 10-cm long 3-micron Microsorb C18 reverse-phase column (Rainin Instrument Co. Inc., Woburn, MA), using a WISP 710B autosampler (Waters, Milford, MA). The mobile phase consisted of 0.15 M monochloroacetic acid buffer (pH 2.9) containing 2 mM EDTA, 0.1 mM 1-octanesulfonic acid sodium salt (Eastman Kodak Co., Rochester, NY) and 12.5% methanol. 5-HTP and 5-HIAA were detected by using an electrochemical detector (model LC-4B; Bioanalytical Systems, Inc., West Lafayette, IN) equipped with a glassy carbon electrode which was set at a potential of +0.6 V (*vs.* Ag/AgCl reference electrode). The concentrations were quantified by comparing the peak heights with those of a known standard. Sample chromatograms are shown in figure 2. Striatal TPH activity from animals treated with 200 μg of THM (fig. 3) was assayed by a $^{14}CO_2$ trapping procedure (Stone *et al.*, 1988).

Determination of TH activity. TH activity was determined according to the method described by Nagatsu (1983). STR and SN were respectively homogenized in 125 and 250 μl of the same homogenizing buffer used in the TPH assay. The homogenates were centrifuged for 15 min at 40,000 × *g* (4°C), and duplicate 10-μl aliquots of the supernatant were 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 tyrosine, 100 nmol of ferrous ammonium sulfate, 320 nmol of *dl*-6-methyl-5,6,7,8-tetrahydrobiopterin, 10 nmol of 2-mercaptoethanol and 20 μmol of sodium acetate. The radiolabeled tyrosine was periodically purified on a column containing Dowex-50 resin (Sigma) and stored in absolute ethanol at

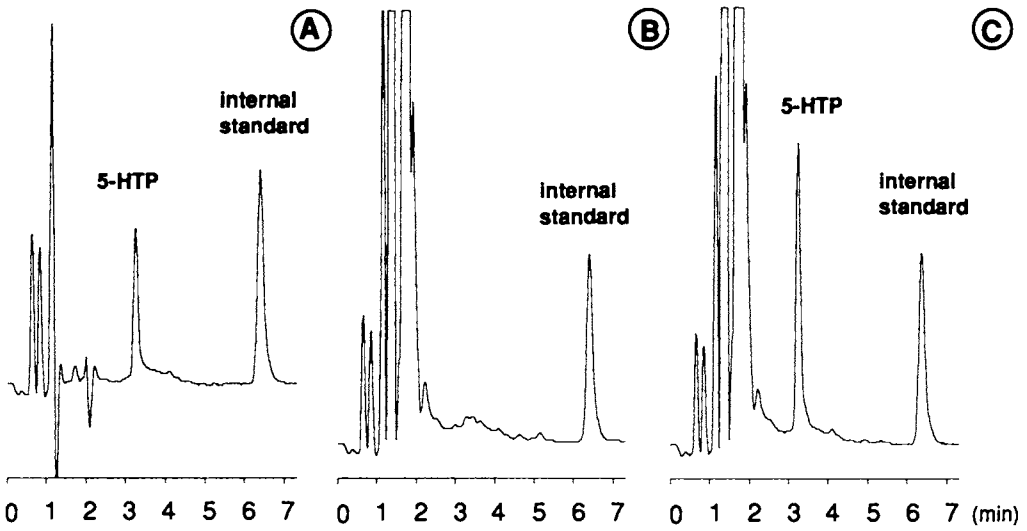


Fig. 2. Chromatographic separation by HPLC of 5-HTP formed in the TPH assay using a 10-cm long 3-micron Microsorb C18 column. A) Chromatogram from a standard solution containing 0.55 ng of 5-HTP and 1.4 ng of 5-HIAA per 5 μl. B) Chromatogram from a blank sample containing boiled cortical TPH. C) Chromatogram from a TPH assay of cortical tissue.

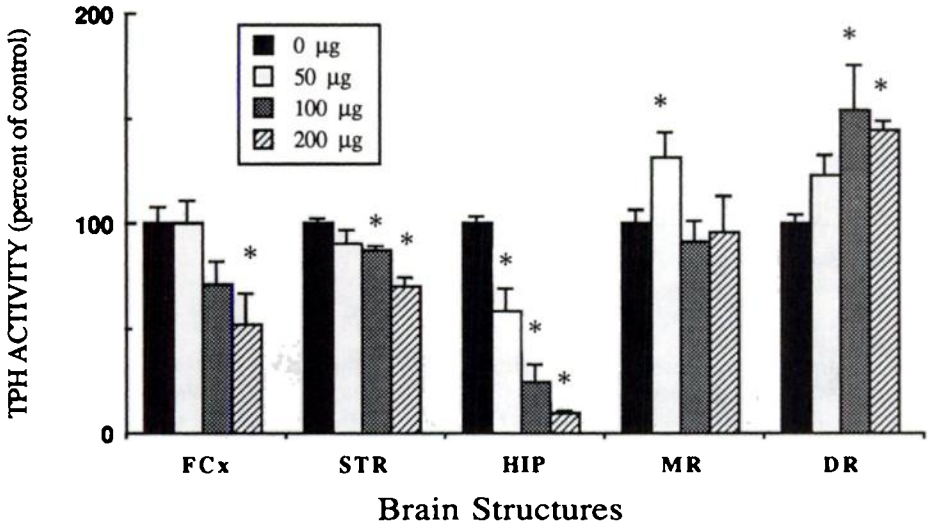


Fig. 3. Effects of different doses of THM on central TPH activity. The rats received one i.c.v. injection of THM (50, 100 or 200 μg THM per 20 μl) or vehicle and were allowed to recover for 5 days. The results are expressed as percent of their respective control (means ± S.E.). The control values for TPH activity, expressed as nmol of hydroxylated tryptophan/h/g tissue, were: FCx, 69.8 ± 5.4; STR, 109.6 ± 4.7; HIP, 143.8 ± 5.2. TPH activity in the MR and in the DR were 6 ± 0.4 and 17.3 ± 0.8 nmol of hydroxylated tryptophan/h/mg protein, respectively (n = 3–11). * $P < .05$ *vs.* 0 μg.

−20°C. The protein content in the SN homogenate was determined using the method of Bradford (1976).

The reaction medium was incubated for 15 min at 37°C, after which the reaction was stopped with 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 2000 × *g* and an aliquot of the supernatant was counted on a liquid scintillation detector (Packard, 2000CA tri-carb, Downers Grove, IL).

Determination of neurotransmitter concentrations. Cortical, striatal and hippocampal 5-HT and 5-HIAA concentrations, as well as striatal DA, DOPAC and HVA concentrations, were determined by HPLC-EC in the side contralateral to the drug injection as reported earlier (Johnson *et al.*, 1988).

Statistical analysis. The results presented in figures 3 to 6 were analyzed with an analysis of variance followed by a Fisher analysis, and the results presented in table 1 were analyzed with a Student's *t* test.

Results

Effects of THM on TPH activity. The effect of 50, 100 or 200 μg of THM on central TPH activity after 5 days is shown in figure 3. Fifty μg of THM decreased HIP TPH activity to 58% of control increasing TPH activity in the MR to 132% of control. Enzymatic activity remained normal in the FCx and in the STR, whereas a trend toward an increase is observed in the DR (123% of control). After 200 μg of THM, TPH activity was reduced to 53, 70 and 10% of control in the FCx, STR and HIP, respectively, whereas a dose of 100 μg gave an intermediate response. The 100- and 200-μg doses of THM increased TPH activity in the DR to 154 and 144% of control, respectively, but failed to alter the enzyme activity in the MR.

Effects of THM on 5-HT and 5-HIAA concentrations. The effect of 50, 100 or 200 μg of THM on contralateral 5-HT and 5-HIAA concentrations after 5 days is shown in figure 4, A and B, respectively. Fifty μg of THM reduced cortical and HIP 5-HT concentrations to 89 and 79% of control, respectively, whereas 100 μg of THM reduced HIP 5-HT concentrations to 60% of control. At 200 μg of THM, 5-HT concentra-

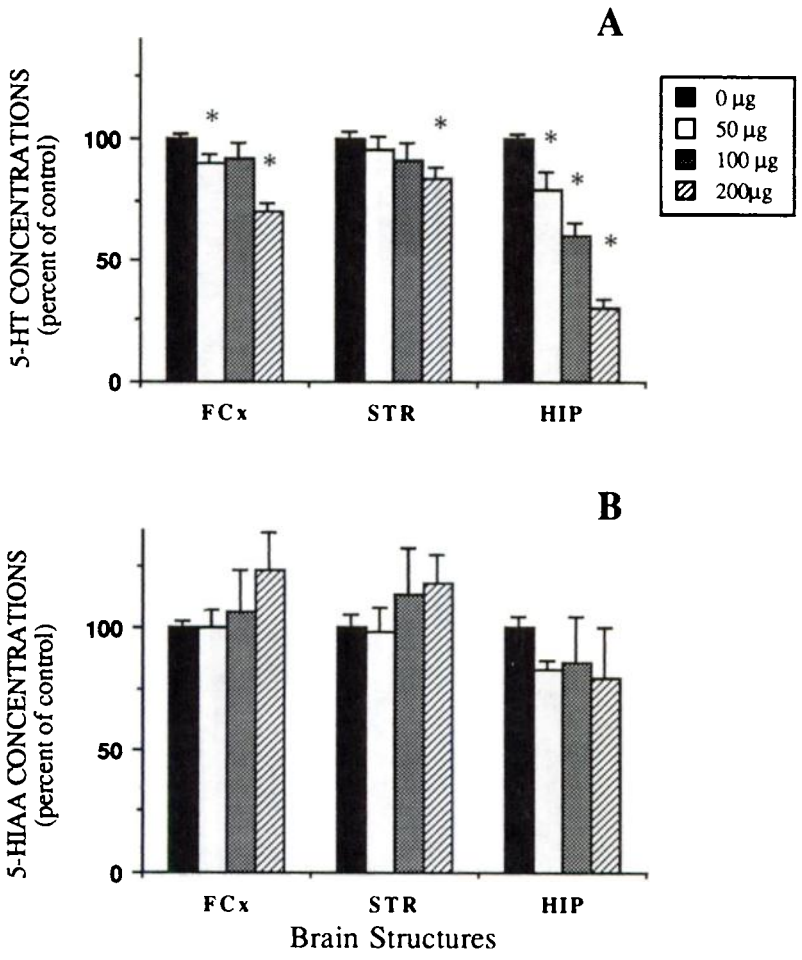


Fig. 4. Effects of different doses of THM on central 5-HT and 5-HIAA concentrations measured contralateral to the side of injection. The animals were treated as described in figure 3. The results are expressed as percent of their respective control (means ± S.E.). The control values for 5-HT concentrations, expressed as µg/g tissue, were: FCx, 0.84 ± 0.02; STR, 0.59 ± 0.02; HIP, 0.45 ± 0.01. 5-HIAA concentrations, expressed as µg/g tissue, were: FCx, 0.24 ± 0.01; STR, 0.50 ± 0.03; HIP, 0.27 ± 0.01 (n = 3–11). * $P < .05$ *vs.* 0 µg.

tions were reduced to 70, 83 and 30% of control in the cortex, STR and HIP, respectively. 5-HIAA concentrations were not statistically different from control in the cortex, STR and HIP (fig. 4B).

Effects of THM on the central dopaminergic system. THM reduced striatal TH activity in a dose-dependent manner, whereas nigral TH activity remained unaltered (fig. 5). THM administered at a dose of 50, 100 or 200 µg reduced striatal TH activity to 76, 56 and 21% of control, respectively. DA, DOPAC and HVA concentrations were also decreased by THM in the striatum (fig. 6). Fifty µg of THM reduced DOPAC and HVA concentrations to 77 and 74% of control, respectively. DA, DOPAC and HVA concentrations were reduced to 36, 44 and 37% of control, respectively, after 200 µg of THM, whereas a dose of 100 µg produced a less extensive decrease.

Effects of DHM on central TPH and TH activity. DHM (135 µg) failed to alter significantly cortical, striatal and hippocampal TPH activity (table 1). However, DHM increased the activity of striatal TH to 115% of control. When higher doses of DHM were tried, all the animals expired.

Discussion

This study demonstrated that THM produced a long-term decline in the activity of TH and TPH, as well as in the

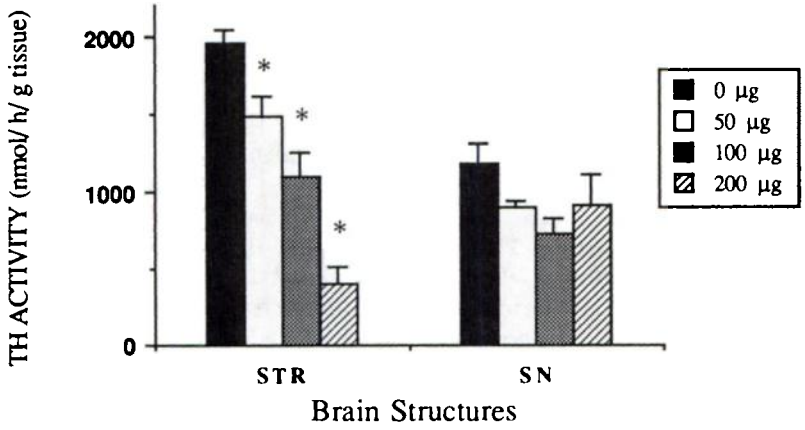


Fig. 5. Effects of different doses of THM on STR and SN TH activity. The animals were treated as described in figure 3. The results are expressed in nmol of hydroxylated tyrosine/h/g tissue (means ± S.E.) (n = 3–11). * $P < .05$ *vs.* 0 µg.

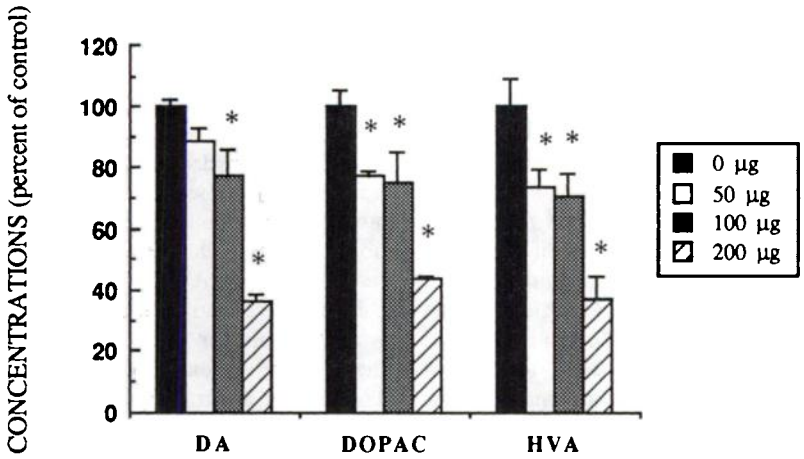


Fig. 6. Effects of different doses of THM on striatal DA, DOPAC and HVA concentrations. The animals were treated as described in figure 3. The results are expressed as percent of control (means ± S.E.). The control values for TPH activity, expressed as μg/g tissue, were: DA, 10.3 ± 0.3; DOPAC, 0.81 ± 0.05; HVA, 0.85 ± 0.08 (n = 3–11). * $P < .05$ *vs.* 0 μg.

TABLE 1

Effect of DHM on brain TPH and TH activities after 5 days

The rats received DHM (135 μg, i.c.v.) and were sacrificed 5 days later. TPH and TH activities are expressed as the mean ± S.E. The number of animals in each group is shown in parentheses.

Brain structures	Control	DHM
	nmol/h/g tissue	
TRH activity		
FCx	95.5 ± 3.5 (7)	91.4 ± 5.0 (7)
STR	30.0 ± 1.8 (6)	24.4 ± 3.7 (7)
HIP	109.3 ± 2.0 (7)	99.6 ± 8.3 (7)
TH activity		
STR	1582 ± 76 (7)	1823 ± 79 (7)*

* $P < .05$ *vs.* control.

concentrations of DA and 5-HT located in nerve terminals after a single i.c.v. administration. This direct effect of THM is in contrast to the lack of effect of centrally administered MDMA. Molliver *et al.* (1986), as well as Paris and Cunningham (1990), observed that MDMA administered locally into the brain produced no long-term neurochemical changes. This indicates that THM may prove useful in elucidating the toxic mechanism whereby MDMA alters brain monoaminergic systems. Interestingly, DHM, the dihydroxy analog of THM, had no effect on the serotonergic system. This difference may be related to the ease by which this catechol oxidizes after addition of a hydroxyl in position 2.

There are some similarities in the neurochemical changes induced by MDMA and THM. MDMA produces a long-lasting decrease in the concentration of 5-HT and in the activity of TPH (Schmidt *et al.*, 1986; Stone *et al.*, 1986, 1987b). These changes are accompanied by a decline in the number of 5-HT reuptake sites, which also suggests the destruction of 5-HT nerve terminals (Battaglia *et al.*, 1987; Commins *et al.*, 1987; Schmidt, 1987). This has been verified by immunocytochemistry visualizing the destruction of the fine axonal structures projecting from the DR (O'Hearn *et al.*, 1988). Interestingly, the axons with large varicosities projecting from the MR are resistant to the action of MDMA. The cell bodies from these two types of 5-HT neurons are not damaged after MDMA treatment. These observations are consistent with the changes induced by THM in the serotonergic system. The ability of THM to reduce TPH activity in brain areas containing serotonergic nerve terminals, but not in structures containing their cell bodies, suggests that THM destroys only nerve endings. However, the possibility that a loss in TPH activity may be masked by an increase in activity in the surviving cells must be considered as well, with the possibility that the destruction of cell bodies may take more than 5 days to occur. A compensation to the loss in enzyme and serotonergic transmission in the nerve terminal region is suggested by the increased TPH activity measured in the DR after 100 and 200 μg of THM. This adaptation would be consistent with the apparent increase in 5-HT release after THM, as suggested by the lack of change in 5-HIAA concentrations in those brain structures showing a decline in 5-HT levels (fig. 4, A and B). Interestingly, the lack of change in TPH activity measured in the MR for two of the three largest THM doses tested coincide with the reported resistance of this serotonergic system to the action of MDMA (O'Hearn *et al.*, 1988).

Our results also demonstrate that THM has properties distinct from those of MDMA because it produces profound alterations in the dopaminergic system (figs. 5 and 6). Multiple administrations of large doses of MDMA fail to alter the concentration of DA or the activity of TH when measured 18 h or 2 weeks after the last drug administration (Stone *et al.*, 1986, 1987a). This is in contrast to the ability of MDMA to produce a neurotoxic response in the serotonergic system after a single administration (Schmidt *et al.*, 1986; Schmidt, 1987). Thus, the decrease in activity of striatal TH and in the concentration of DA after THM clearly differs from MDMA and may be attributed to the structural similarity of 6-hydroxydopamine and THM (fig. 1). Presently, our results suggest that THM toxicity is more like that of methamphetamine, an MDMA analog, which alters both the dopaminergic and serotonergic systems (Hotchkiss *et al.*, 1979).

Apparently, 6-hydroxydopamine differs from THM in its ability to destroy dopaminergic cell bodies. Intracerebroventricular administration of 6-hydroxydopamine was reported to destroy nigral cell bodies within 2 days of administration (Hedreen, 1978), although others failed to see a change at such an early time (Constantinidis *et al.*, 1971). Onn *et al.* (1986) observed that the complete loss of TH-positive cells in the SN requires more than 2 weeks after intraventricular injection of 6-hydroxydopamine. The results presented in figure 5 suggest

that THM does not kill dopaminergic cell bodies. However, an effect of THM on nigral TH activity could become apparent over a period of 2 weeks. Thus, our results do not allow us to identify with certainty a difference in properties between THM and 6-hydroxydopamine.

It is important to note that THM is uniquely different from MDMA and the other amphetamine analogs because it induces long-term alterations in monoaminergic systems after i.c.v. injection. Intracerebroventricular administration of amphetamine analogs fails to induce the neuronal damage and biochemical alterations observed after the peripheral administration of the drugs (Berger *et al.*, 1990; Gál *et al.*, 1975; Molliver *et al.*, 1986; Paris and Cunningham, 1990). Thus, until now, it has been difficult to study the central toxicity of amphetamine analogs because it was impossible to determine whether the disturbances generated in the periphery have an impact on the central response. For instance, we reported that corticosterone and adrenalectomy modify the MDMA-induced changes in hippocampal TPH activity (Johnson *et al.*, 1989). Because of its central action, THM is a useful tool for studying MDMA toxicity. Use of THM will require intracranial injection because the polarity of the compound may prevent it from crossing the blood-brain barrier after its injection in the periphery.

The magnitude of the decrease in TPH activity after THM varied in the FCx, STR and HIP (fig. 3). The hippocampal serotonergic system was most sensitive to the effect of THM. The greater sensitivity of the hippocampal serotonergic system compared to the FCx may be explained by the proximity of the lateral ventricles to the HIP, thus permitting greater access to THM. However, this difference in exposure to THM would not explain the lower sensitivity of the striatal system because it is also close to the ventricles. We are currently investigating the brain distribution of THM in an attempt to explain this discrepancy. Another explanation might be that the hippocampal serotonergic system is more susceptible to the action of THM than is the serotonergic system of other brain areas. It is also interesting that THM induced a greater decline in striatal TH activity compared to striatal TPH activity. This suggests that the striatal dopaminergic system may be more vulnerable to THM than the serotonergic system. This observation is reminiscent of the greater vulnerability of the dopaminergic system to 6-hydroxydopamine (Kostrzewa and Jacobowitz, 1974). Preferential uptake of THM into dopaminergic nerve terminal could explain such a difference. This is not unexpected in view of the presence of the catechol moiety in the chemical structure of THM.

The neurochemical alterations induced in TH and TPH activities by THM are not produced by DHM. Because this catecholamine metabolite of MDMA is closely related to THM, it is unlikely that the difference could be explained by access to different compartments. Our results confirm the recent report by Steele *et al.* (1991), who demonstrated that 5-HT and DA concentrations were unaltered by DHM. McCann and Ricaurte (1991) also reported that α-methyl-DA, the N-demethylated analog of DHM, is also devoid of neurotoxic properties. Contrary to Steele *et al.*, we were unable to investigate DHM at higher concentrations because of the high mortality observed at these doses within 6 h of administration. The discrepancy in tolerance to the drug may be due to the presence of anesthetics when we injected DHM. The results on this metabolite in table 1 allow some comparisons with THM because this compound produced alterations at doses equivalent to or lower than the dose used for DHM. The lack of change in the dopaminergic and serotonergic systems after DHM stresses the critical role of the hydroxy group in position 2 in THM, and indicates that the mechanism of action of THM may be similar to 6-hydroxydopamine, which involves the generation of quinones, hydrogen peroxide and reactive radicals (Kostrzewa and Jacobowitz, 1974). Thus, access of THM into the nerve terminal followed by its auto-oxidation and the generation of reactive chemicals may be a prerequisite for producing the loss in enzyme activity measured in this study.

From these results, it is not possible to conclude that THM is responsible for the MDMA-induced neurotoxicity because the drugs produce different effects in the dopaminergic system. The lack of a significant role for THM in MDMA toxicity is also suggested, as we and others failed to induce neurotoxicity by i.c.v. administration of 6-hydroxy-MDMA, the precursor of THM and a metabolite of MDMA (Zhao *et al.*, 1990, personal results). Moreover, recent evidence suggests that a mechanism other than the generation of a metabolite may be responsible for MDMA toxicity because administration of a 5-HT_2 receptor antagonist, or the depletion of central DA, prevents MDMA-induced toxicity (Schmidt *et al.*, 1990, 1991; Stone *et al.*, 1988). However, THM cannot be totally excluded as a causative agent in MDMA-induced neurotoxicity because 6-hydroxy-MDMA can be metabolized to THM (Lim and Foltz, 1991b; Lim *et al.*, 1991b). The selective neurotoxic effect of MDMA could result from the generation of THM exclusively in serotonergic nerve terminals. Moreover, 5-HT_2 receptor antagonists and central DA depletion may exert a protective effect by preventing the conditions favoring the metabolism of MDMA into THM.

In summary, THM, but not DHM, produced a long-term decline in activity of TH and TPH as well as in the concentrations of DA and 5-HT after a single i.c.v. administration. The dopaminergic system was more sensitive than the serotonergic system while the enzymatic activity in the cell bodies of the two transmitter systems remained unaltered after THM. Although THM was not established as the neurotoxic metabolite explaining MDMA toxicity, we demonstrated the possibility that this metabolite could account for some of MDMA properties. It is possible that other substances analogous to THM could be generated from the metabolism of other amphetamine analogs and participate in their neurotoxic effects.

Acknowledgments

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