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EFFECTS OF *N*-ETHYL-3,4-METHYLENEDIOXYAMPHETAMINE (MDE) ON CENTRAL SEROTONERGIC AND DOPAMINERGIC SYSTEMS OF THE RAT

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Abstract—The influence of *N*-ethyl-3,4-methylenedioxyamphetamine (MDE) on the central serotonergic and dopaminergic systems of the rat after a single or multiple injections was studied. MDE (10 mg/kg) produced a significant decrease in the concentration of 5-hydroxytryptamine (5-HT) 1 hr later in the frontal cortex and the hippocampus without affecting the concentration of 5-hydroxyindoleacetic acid (5-HIAA) or tryptophan hydroxylase (TPH) activity. Hypothalamic and neostriatal concentrations of 5-HT, 5-HIAA, dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) remained unaffected, as well as the neostriatal TPH and tyrosine hydroxylase (TH) activities. However, 3 hr after the MDE injection, the serotonergic variables including TPH activity were decreased in most of the brain areas examined. The dopaminergic system remained unaffected, except for a significant reduction in neostriatal DOPAC concentrations. The changes in transmitter concentrations after a single injection were dose dependent; the maximum depletion in TPH activity was reached with a 10 mg/kg dose. The administration of multiple doses of MDE caused greater decreases in TPH activity and 5-HT concentrations 3 hr after the treatment than did a single injection; in addition, a partial recovery from multiple administrations occurred by 18 hr. The effects of MDE on DA and its metabolites were transient, and neostriatal TH activity was not altered. This study demonstrates that MDE primarily affects the central serotonergic system, as reported for its congeners 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine. It does, however, produce less neurotoxicity as judged by its lower potency on the dopaminergic and the serotonergic systems as well as the recovery occurring in these systems.

3,4-Methylenedioxyamphetamine (MDA), a synthetic psychoactive agent, has been investigated for its psychotomimetic properties, its potential therapeutic actions in the treatment of Parkinson's disease, as well as for its anorexic effects [1]. The abuse of MDA and its congeners has recently received renewed interest, but there is relatively little reported about their properties. MDA and its N-methylated congener, 3,4-methylenedioxymethamphetamine (MDMA), are potent releasers of central 5-hydroxytryptamine (5-HT) [2, 3] and dramatically decrease the concentrations of this transmitter and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the rat cortex, hippocampus and neostriatum following acute or multiple injections of either drug [4]. Both compounds decrease the activity of the 5-HT-synthesizing enzyme, tryptophan hydroxylase (TPH), in a manner similar to *p*-chloroamphetamine (PCA) [5].

The N-methylation of racemic MDA to MDMA appears to attenuate the ability of this compound to alter the serotonergic system in the rat brain; moreover, in contrast to MDA, a single dose of MDMA increases the neostriatal concentration of the dopamine (DA) metabolite, homovanillic acid (HVA) [4]. Interestingly, N-methylation of amphetamine to methamphetamine (METH) actually enhances its ability to decrease central 5-HT concentrations [6].

Another MDA analogue, *N*-ethyl-3,4-methylenedioxyamphetamine (MDE), is slightly less potent than MDA as an analgesic [7], is more potent in its ability to induce motor activity and has psychotomimetic activity similar to MDMA [1, 7], although its duration of action tends to be shorter than that of its analogues [1]. Presently, there is limited information about the effects of MDE on central biogenic amines. The present study examines the effects of single or multiple administrations of MDE on the concentrations of 5-HT and DA, their metabolites and their respective biosynthetic enzymes, tryptophan hydroxylase (TPH; tryptophan 5-monooxygenase, EC 1.14.16.4) and tyrosine hydroxylase (TH; tyrosine 3-monooxygenase, EC 1.14.16.2).

METHODS

Male Sprague–Dawley rats (180–250 g) were housed six per cage in a room with controlled lighting (12-hr light/dark cycle) and heating (24°). Rats had access to food and water *ad lib*.

Treatment and dissection. In the acute treatment, animals were injected subcutaneously with (±)-MDE hydrochloride (supplied by the National Insti-

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tute on Drug Abuse) or 0.9% saline and killed 1 or 3 hr later. In the multiple dose studies, animals were given five injections of MDE (10 mg/kg) at 6-hr intervals and killed either 3 or 18 hr after the last injection. The doses of MDE used in this study are expressed as the free base. After decapitation, the brain was quickly removed and placed on a cold plate. Frontal cortex (corresponding to the pregenual part of the anteromedial cortex described by Emson and Lindvall [8]), neostriatum, hypothalamus and hippocampus were removed and frozen on dry ice. All tissues were stored at −80° until assayed.

Tyrosine hydroxylase (TH) assay. TH activity was measured according to the method described by Nagatsu [9]. Briefly, neostriata were weighed and homogenized in 125 μl of 5 mM dithiothreitol (Calbiochem-Behring Corp., San Diego, CA), 50 mM *N*-2-hydroxyethylpiperazine - *N'* - 2 - ethanesulfonic acid (HEPES) buffer (Sigma Chemical Co., St. Louis, MO) (pH 7.4) containing 0.2% (v/v) Triton X-100 and centrifuged for 15 min at 40,000 *g* (4°). Duplicate 10-μl aliquots were added to 40 μl of double-distilled water and assayed for TH activity using (±)-6-methyl-5,6,7,8-tetrahydrobiopterin (Sigma Chemical Co.) as cofactor. Each incubation medium contained 550,000 dpm of [3,5-^{3}H]tyrosine (54.2 Ci/mmol, New England Nuclear Research Products, Boston, MA). The final volume of the reaction medium was 100 μl and contained 10 nmol tyrosine, 100 nmol ferrous ammonium sulfate, 320 nmol (±)-6-methyl-5,6,7,8-tetrahydrobiopterin, 10 nmol 2-mercaptoethanol, and 20 μmol sodium acetate. The radiolabeled tyrosine was periodically purified on a column containing Dowex-50 resin (Sigma Chemical Co.) and stored in absolute ethanol (−20°) until the assay [9].

The reaction medium was incubated for 15 min at 37° after which the reaction was stopped with the addition of 100 μl of 10% trichloroacetic acid. The acidified reaction mixture was transferred onto a Dowex-50 column (0.5 × 2 cm). Each tube was rinsed with 0.9 ml water and transferred to the appropriate column after which an additional 0.9 ml of water was added to each column. The total effluent was collected in a scintillation vial to which 10 ml of scintillation fluid was added.

Tryptophan hydroxylase (TPH) assay. Frontal cortex, hippocampus and neostriatum were homogenized in 80, 200 and 125 μl, respectively, of 5 mM dithiothreitol, 50 mM HEPES buffer (pH 7.4) containing 0.2% (v/v) Triton X-100 and centrifuged for 15 min at 40,000 *g* (4°). Duplicate 7.5-μl aliquots of the supernatant fraction were used to measure TPH activity by a modified $^{14}CO_2$ trapping procedure [10, 11] using (±)-6-methyl-5,6,7,8-tetrahydrobiopterin as cofactor. Each reaction mixture contained 21,000 dpm of [side chain-1-^{14}C]tryptophan (50 mCi/mmol; New England Nuclear Research Products). Details of the method are reported by Hotchkiss *et al.* [12].

Assays for monoamines and metabolites. DA, 5-HT and their metabolites dihydroxyphenylacetic acid (DOPAC), HVA, and 5-HIAA were measured using high-performance liquid chromatography (HPLC) with electrochemical detection (model LC-4B, Bioanalytical Systems Inc., West Lafayette, IN) according to a modification of the method described by Nielsen and Moore [13]. Tissues were weighed, homogenized in a 0.15 M monochloroacetic acid buffer (pH 2.9) containing 2 mM EDTA, 0.1 mM 1-octanesulfonic acid sodium salt and 12.5% methanol, and centrifuged at 4000 *g* for 15 min (4°). Supernatant fractions were filtered with a 0.2-μm Microfilter system (Bioanalytical Systems Inc.) and 50 μl was injected with an autosampler (WISP model 710B, Waters, Milford, MA) onto a 10-cm Microsorb (3 μm) reversed-phase column (Rainin Instrument Co., Woburn, MA). The mobile phase consisted of the same buffer in which brain tissue was homogenized. The eluent was monitored using a glassy carbon electrode with the potential set at +0.73 V (vs Ag/AgCl reference electrode), and the data were recorded on an RYT double pen recorder (Bioanalytical Systems Inc.).

Statistics. Differences between group means were analyzed by the two-tailed Student's *t*-test and were considered significant when the P value was less than 0.05. A one-way ANOVA analysis with a Student–Newman–Keuls multiple comparison test was used to analyze the data in Figs. 4–6.

RESULTS

The effects of MDE were less pronounced 1 hr after a single injection than in a later time. One hour after a single injection of MDE (10 mg/kg), the concentrations of 5-HT were decreased significantly in the frontal cortex and hippocampus to 67 and 65% of control, respectively, whereas 5-HT concentrations in hypothalamic and neostriatal areas remained unaltered (Table 1). Concentrations of 5-HIAA remained unaffected in all brain regions examined, as did the concentrations of DA, DOPAC or HVA in the hypothalamus and neostriatum (Table 1).

The dose–response relationships for the effects of MDE on the concentrations of 5-HT (Fig. 1), DA (Fig. 2) and their metabolites were examined 3 hr after MDE administration. The only effect of a 5 mg/kg dose of MDE was a decline in the hippocampal 5-HT concentration to 78% of control. The higher doses of MDE (10 and 20 mg/kg) caused significant, dose-dependent decreases in 5-HT concentrations in all tissues analyzed (frontal cortex, hippocampus, hypothalamus and neostriatum). Although the higher doses of MDE did decrease significantly the cortical and neostriatal 5-HIAA levels, these changes were smaller than those in the corresponding 5-HT concentrations. Interestingly, the response of the serotonergic system in the hypothalamus was somewhat distinct from the other brain structures as the declines in the concentrations of 5-HIAA were approximately the same as the decreases in the concentrations of 5-HT.

In contrast to the serotonergic system, the response of dopaminergic systems of the hypothalamus and neostriatum were considerably less dramatic. No changes in the concentrations of DA or HVA were seen following any of the MDE doses in either brain region. The only significant change was a dose-dependent decrease in the neostriatal levels of DOPAC.

Table 1. Effects of MDE on monoaminergic transmitters 1 hr following an acute administration

	Transmitter concentrations (μg/g tissue)		
	Control	MDE (10 mg/kg)	% Control
Frontal cortex			
5-HT	0.66 ± 0.02 (6)	0.44 ± 0.04* (6)	67
5-HIAA	0.23 ± 0.02 (6)	0.26 ± 0.02 (6)	113
Hippocampus			
5-HT	0.48 ± 0.04 (6)	0.31 ± 0.02† (6)	65
5-HIAA	0.36 ± 0.03 (6)	0.33 ± 0.02 (6)	92
Hypothalamus			
5-HT	0.70 ± 0.08 (5)	0.78 ± 0.04 (6)	111
5-HIAA	0.33 ± 0.05 (5)	0.34 ± 0.02 (6)	103
DA	0.31 ± 0.02 (4)	0.38 ± 0.03 (6)	123
DOPAC	0.028 ± 0.006 (4)	0.048 ± 0.009 (6)	171
Striatum			
5-HT	0.43 ± 0.02 (6)	0.43 ± 0.03 (6)	100
5-HIAA	0.42 ± 0.03 (6)	0.48 ± 0.01 (6)	114
DA	8.58 ± 0.66 (6)	9.56 ± 0.66 (6)	111
DOPAC	0.74 ± 0.07 (6)	0.64 ± 0.06 (6)	87
HVA	0.75 ± 0.05 (6)	0.60 ± 0.03 (6)	80

Transmitter concentrations are means ± SE; the number of determinations is in parentheses. Rats were killed 1 hr after a single injection of MDE (10 mg/kg). The value of the MDE group in percent of the respective control is shown in the percent column. Statistical analysis was performed with a Student's *t*-test.

* $P < 0.001$.

† $P < 0.01$.

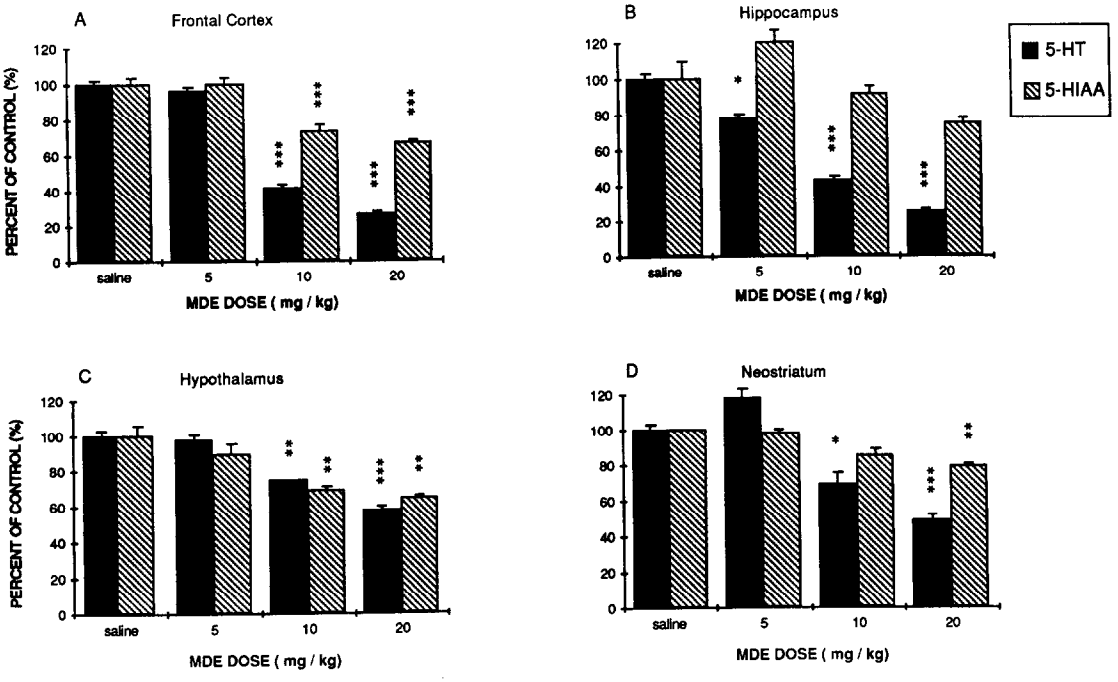

Fig. 1. Dose–response relationship of MDE on 5-HT and 5-HIAA concentrations. Rats were injected subcutaneously with either saline or saline plus MDE (5, 10 or 20 mg/kg) and killed 3 hr later. The means of the 5-HT and 5-HIAA concentrations in the frontal cortex (A), hippocampus (B), hypothalamus (C) and neostriatum (D) are expressed in percentage of control (saline) ±SE. SE is not shown when the value is less than 1.5% of control. Means ± SE of 5-HT concentrations in the saline-treated rats, expressed as μg/g tissue, were: 0.53 ± 0.02, frontal cortex; 0.40 ± 0.02, hippocampus; 1.0 ± 0.06, hypothalamus; and 0.45 ± 0.02, neostriatum. Concentrations of 5-HIAA were: 0.15 ± 0.01 in the frontal cortex; 0.24 ± 0.04 in the hippocampus; 0.39 ± 0.04 in the hypothalamus; and 0.48 ± 0.01 in the neostriatum. Statistical analyses were performed with a Student's *t*-test to compare concentrations of 5-HT or 5-HIAA with control groups. Key: *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$ ($N = 5$ or 6).

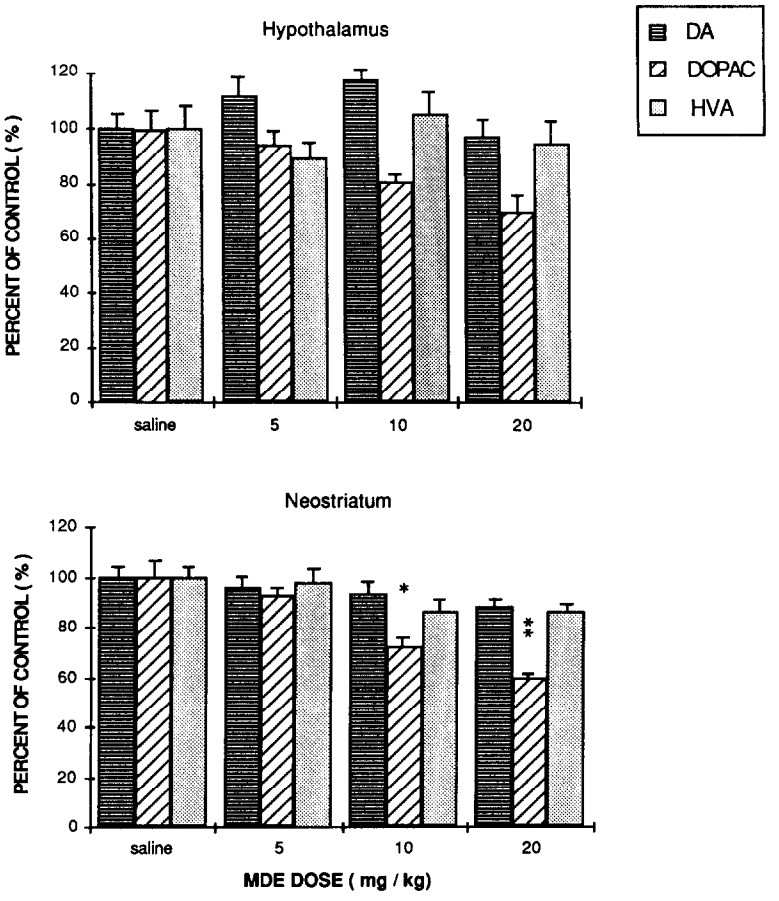

Fig. 2. Effects of various doses of MDE on hypothalamic and neostriatal concentrations of DA, DOPAC and HVA. Animals were treated as described in the legend of Fig. 1. The means ± SE are expressed as a percentage of control (saline). Concentrations of DA in the saline-treated rats (μg/g tissue ± SE) were: 0.33 ± 0.03 in the hypothalamus and 10.27 ± 0.64 in the neostriatum. The concentrations for DOPAC and HVA were: 0.036 ± 0.004 and 0.020 ± 0.003 in the hypothalamus while these concentrations were 0.99 ± 0.12 and 0.62 ± 0.05 in the neostriatum. Statistical analyses were performed with a Student's *t*-test to compare concentrations of DA or its metabolite with the control group. Key: *$P < 0.05$; and **$P < 0.01$ ($N = 5$ or 6).

One hour after a single injection of MDE (10 mg/kg), TPH activity was unaltered in the frontal cortex, hippocampus and neostriatum (Fig. 3). Three hours following a 5 mg/kg dose of MDE, the TPH activity was decreased significantly (74% of control) in the hippocampus, while decreases in TPH activity following the 10 and 20 mg/kg doses were approximately the same in the hippocampus (~60% of control), frontal cortex (~75% of control) and neostriatum (~70% of control).

The 5-HT and metabolite concentrations 3 and 18 hr after five successive doses (6-hr intervals) of MDE (10 mg/kg) are described in Fig. 4. This treatment depleted 5-HT and 5-HIAA concentrations in all brain areas examined 3 hr following the last dose of drug. 5-HT concentrations ranged from 23 to 60% of control, while the 5-HIAA levels ranged from 41 to 53% of control. In all areas, significant recovery of 5-HT and 5-HIAA concentrations was observed 18 hr after treatment. 5-HT concentrations ranged from 60 to 84% of control, whereas all 5-HIAA concentrations had returned to control levels. Interestingly, the neostriatal DOPAC and HVA concentrations were decreased significantly 3 hr after the last MDE dose (46 and 67% of control respectively), but the concentrations of these compounds were not altered in the hypothalamus at this time point (Fig. 5). Neostriatal DA showed a trend toward lower concentrations at the 3-hr time. By 18 hr after the last dose, neostriatal DA, DOPAC and HVA concentrations had returned to control levels.

The TPH activity was decreased 3 hr after multiple doses of MDE (10 mg/kg) in the frontal cortex, hippocampus and neostriatum (47, 39 and 60% of control respectively; Fig. 6). Determination of the TPH activity 18 hr after the last MDE injection demonstrates a return toward normal enzymatic activity in all tissues, although it was still significantly lower than corresponding controls. In contrast, the neostriatal TH activity after either a single or multiple injections of MDE remained unaffected at all times and doses examined (Table 2).

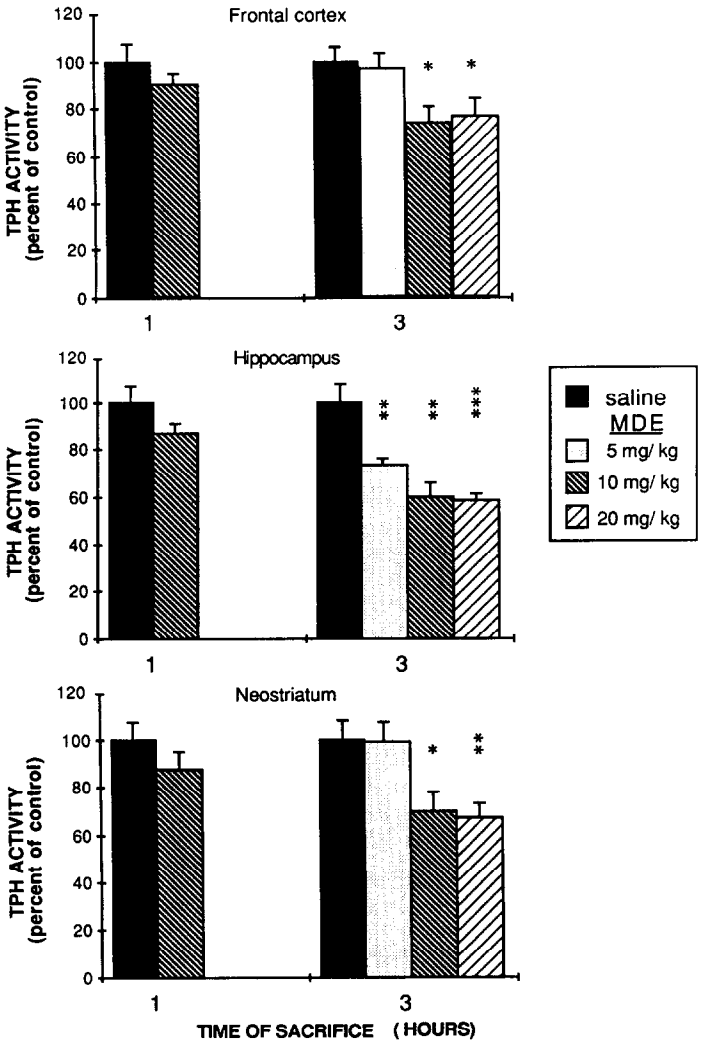

Fig. 3. Effects of MDE (10 mg/kg) 1 hr after a single injection and of MDE (5, 10 or 20 mg/kg) 3 hr after the injection on the frontal cortex, hippocampal and neostriatal TPH activity. The enzymatic activities are expressed as percent ± SE of the control group (injected with saline) for the respective time of sacrifice. Enzymatic activities of the control groups (3 hr), expressed in nmol of hydroxylated tryptophan/hr/g of tissue, were: 68·0 ± 4.3 in the frontal cortex; 52.0 ± 4.5 in the hippocampus; and 49.5 ± 4.0 in the neostriatum. Statistical analyses of the enzymatic activity between means of the MDE groups and control were performed with Student's *t*-test. Key: *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$ ($N = 6$).

Table 2. Effects of MDE on neostriatal TH activity

Treatment	Dose of MDE (mg/kg)	TH activity (nmol/hr/g tissue) Control	MDE	Control
1 hr after single injection	10	2893 ± 201 (6)	3096 ± 312 (6)	107
3 hr after single injection	5	2702 ± 122 (6)	2492 ± 225 (6)	91
	10		2646 ± 252 (6)	98
	20		2680 ± 199 (6)	99
3 hr after the fifth injection	10	2525 ± 243 (6)	2702 ± 265 (6)	107
18 hr afer the fifth injection	10	2986 ± 203 (12)	2810 ± 186 (14)	94

Details of the different treatments are described in Methods. Means ± SE of TH activity are expressed in nmol of hydroxylated tyrosine per hr per g of tissue. The number of determinations is shown in parentheses. Each mean value is expressed as percent of control in the last column.

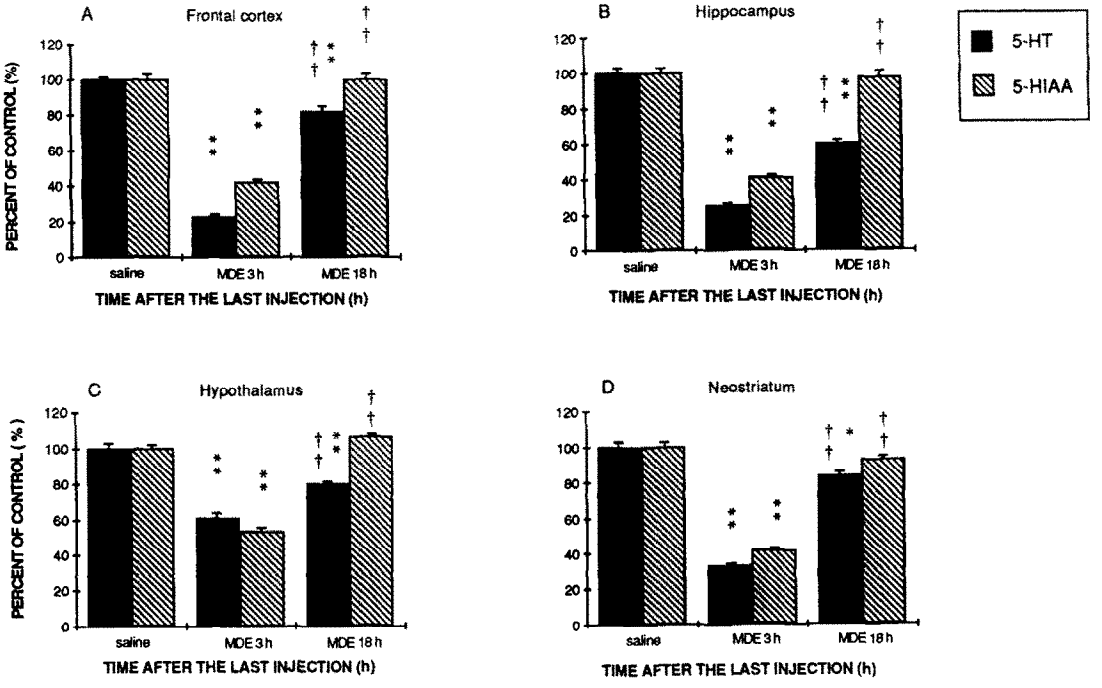

Fig. 4. Effects of multiple administrations of MDE (10 mg/kg) on 5-HT and 5-HIAA concentrations 3 and 18 hr after treatment. The means of the 5-HT and 5-HIAA concentrations in the frontal cortex (A), hippocampus (B), hypothalamus (C) and neostriatum (D) are expressed in a percentage of control (saline) ± SE. As there was no significant difference between the two control groups at each time of sacrifice, these determinations were combined and expressed as a single control group in order to simplify the figure. Means of 5-HT concentrations of the control (saline) group, expressed as μg/g tissue, were: 0.62 ± 0.01 in the frontal cortex; 0.40 ± 0.02 in the hippocampus; 0.86 ± 0.04 in the hypothalamus; and 0.43 ± 0.02 in the neostriatum. Concentrations of control 5-HIAA were: 0.19 ± 0.01 in the frontal cortex; 0.32 ± 0.02 in the hippocampus; 0.45 ± 0.02 in the hypothalamus; and 0.41 ± 0.02 in neostriatum. Statistical analyses were performed with a one-way ANOVA test, while a Student–Newman–Keuls test was used for the multiple comparisons analysis. Key: $^{*}P < 0.05$; and $^{**}P < 0.01$ versus respective control; and $^{\dagger\dagger}P < 0.01$ versus corresponding 3-hr group (N = 16–18 for control; N = 6 for the 3-hr group; N = 13–14 for the 18-hr group).

DISCUSSION

The results from this study demonstrate that MDE profoundly affects several central serotonergic systems while having substantially less impact on dopaminergic pathways. However, the MDE-induced changes in the 5-HT-related variables, while highly significant, were less robust and more transient than those resulting from the administration of its analogues, MDA and MDMA. Stone *et al.* [4] reported a 50% decrease of neostriatal TPH activity 3 hr following a single injection (10 mg/kg) of racemic MDA or MDMA, while we observed a 30% decrease after similar treatment with MDE (Fig. 3). Comparisons between these two studies demonstrate that equal doses of MDA and MDMA cause substantially greater reductions than MDE in the concentrations of 5-HT and 5-HIAA in neostriatum, hippocampus and frontal cortex.

Interestingly, we found that 18 hr following multiple MDE administrations, there was considerable recovery of the serotonergic variables in all brain areas examined. This is in sharp contrast to the effects of multiple administrations of similar doses of MDA or MDMA where there are no signs of recovery in the depressed serotonergic systems after this time period [4]; in fact, the effects appear to persist for at least 2 weeks [14]. Relevant to these data are the findings by Ricaurte *et al.* [14] that neuronal cell damage occurs after multiple doses of MDA (10 mg/kg) accompanied by a decrease of synaptosomal 5-HT uptake, suggesting that drug-induced changes in the serotonergic variables reflect neurotoxic actions of these MDA-related compounds. If this hypothesis is correct, the apparent rapid recovery of the 5-HT systems, which occurs following MDE administration, implies that this agent is less neurotoxic than its analogues, MDA and MDMA. As the TPH activity and 5-HT concentration are still slightly decreased in some brain areas 2 weeks after a multiple dose treatment with MDE, it is apparent that this designer drug may have some neurotoxic properties [15]. This lower potency of MDE is consistent with the decrease of central activity reported for the *N*-ethyl analogs of amphetamine and MDA [1, 16].

The mechanism responsible for the serotonergic changes, mediated by MDE or by MDA and MDMA, is unknown. Although amphetamine injection decreases TPH activity, it does not reduce TPH

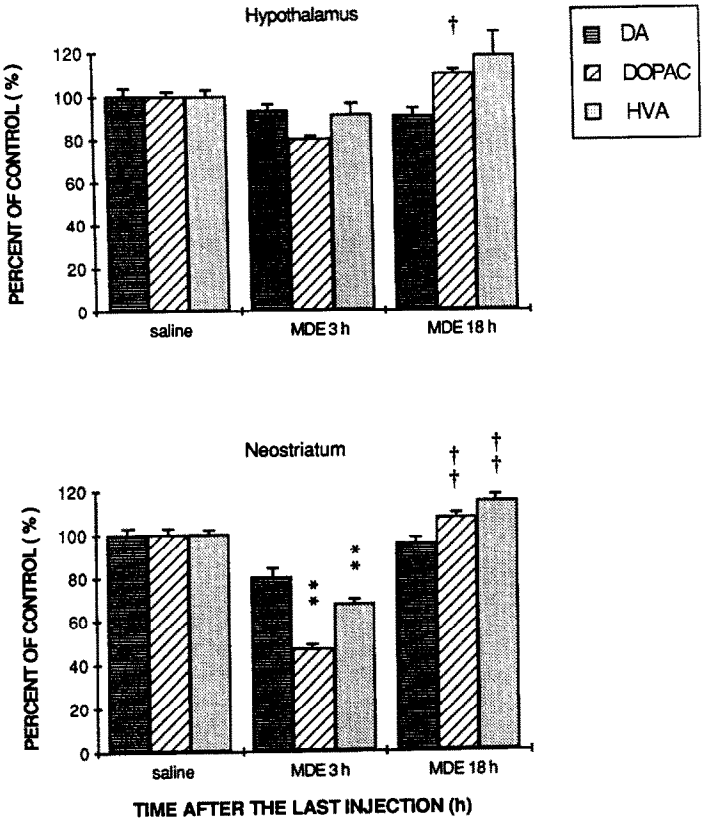

Fig. 5. Effects of multiple administrations of MDE (10 mg/kg) on hypothalamic and neostriatal concentrations of DA, DOPAC and HVA 3 and 18 hr after treatment. Means ± SE are expressed as percentage of control (saline). Concentrations of DA (μg/g tissue) in the saline-treated rats were: 0.32 ± 0.02 in the hypothalamus and 9.07 ± 0.45 in the neostriatum. The concentrations for DOPAC and HVA were: 0.050 ± 0.002 and 0.022 ± 0.002 in hypothalamus, whereas these concentrations were 0.91 ± 0.05 and 0.61 ± 0.02 in the neostriatum. Statistical analyses were performed with a one-way ANOVA test, while a Student–Newman–Keuls test was used for the multiple comparisons analysis. Key: $^{**}P < 0.01$ versus control; and $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$ versus the corresponding 3-hr group (N values were same as for Fig. 4).

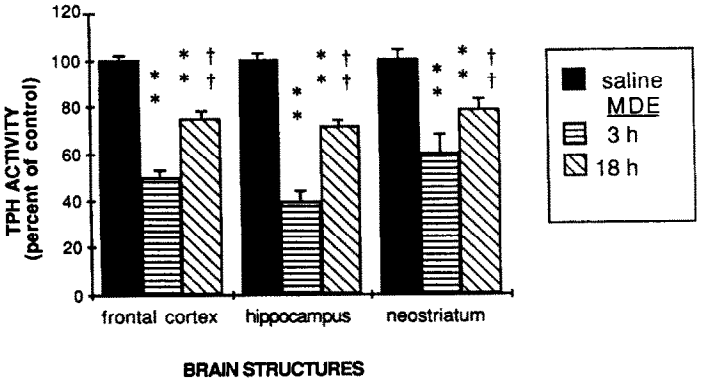

Fig. 6. Effects of multiple injections of MDE (10 mg/kg) 3 and 18 hr after treatment on TPH activity in various brain regions. Means ± SE are expressed as a percentage of the corresponding control (saline). Enzymatic activities of the control, expressed in nmol hydroxylated tryptophan/hr/g tissue, were: 64 ± 1.6 in the frontal cortex; 60.5 ± 1.9 in the hippocampus; and 48.3 ± 1.6 in the neostriatum. Statistical analyses on the enzymatic activity were performed with a one-way ANOVA test, while a Student–Newman–Keuls test was used for the multiple comparisons analysis. Key: $^{**}P < 0.01$ versus control; and $^{\dagger\dagger}P < 0.01$ versus the corresponding 3-hr group (N values were same as for Fig. 4).

activity *in vitro* [17]; it is therefore unlikely that the reduction in the enzyme activity measured in this study could be explained by the direct inhibitory action of the amphetamine-like compound on TPH. It is interesting that the toxic effects of MDA and MDMA resemble those produced by *p*-chloroamphetamine (PCA) and methamphetamine (METH) in their ability to cause long-term declines in TPH activity, 5-HT concentrations, synaptosomal uptake of 5-HT [5, 18, 19] and cellular neurotoxicity [18–21]. Moreover, toxic effects on the serotonergic systems by PCA, METH or MDMA can be prevented with 5-HT uptake blockers [22–24]. This similarity suggests a common mechanism of action of these amphetamine-like compounds.

It is possible that some neurotoxic substance generated by these compounds requires entry into 5-HT neurons via the active transport mechanism in order to mediate their effects. The fact that PCA, METH, and MDMA are lipophilic and diffuse freely into neuronal terminals without the aid of an uptake system [3, 25–27] argues against the possibility that these drugs are themselves the neurotoxic agent. It has been proposed that metabolites of these amphetamine-like drugs [28] or that drug-induced release of DA [29] causes the neurotoxicity. Without additional information, little can be concluded about the neurotoxic actions of these drug metabolites; however, considerable work has been reported concerning the neurotoxic potential of DA which could have implications relative to serotonergic systems because of the ability of 5-HT uptake mechanisms to transport DA intraneuronally [29]. Relevant to the present discussion are our previous observations that inhibition of DA synthesis with alpha-methyl-*p*-tyrosine protects the 5-HT system from the toxic effects of METH administered either acutely or by multiple doses [29, 30]; the toxic effects of METH reappear when DA synthesis inhibition by alpha-methyl-*p*-tyrosine is circumvented with the addition of the DA precursor, dihydroxyphenylalanine (DOPA) [29]. It has been suggested that METH-released DA is oxidized to the toxic compound, 6-hydroxydopamine, which in turn is responsible for METH-induced neuronal damage [31]. However, the role of 6-hydroxydopamine still remains to be established since Rollema *et al.* [32] failed to detect this neurotoxin in the neostriatum of rats treated with METH.

While the above findings implicate oxidized DA as a participant in the catecholaminergic neuron impairment mediated by METH, there are some problems with invoking the same mechanisms for the serotonergic-related neurotoxicity of MDA, MDMA and MDE. The effects of the MDA analogues resemble those of PCA, a drug reported to have less effect on catecholaminergic systems [33], than METH. Thus, while the MDA analogues do affect dopaminergic variables (Figs. 2 and 5) [4], these are much less intense than those following treatment with METH [3, 4]. However, it is noteworthy that MDA and MDMA do cause DA release from striatal tissue slices [3] and, interestingly, the least neurotoxic drug, MDE, is also the least effective DA releaser of the analogues.* In contrast, the neurotoxic potencies of the drugs do not correspond well with their ability to evoke 5-HT release; all three compounds are approximately equipotent in this releasing capability.* Thus, the observations from this study on MDE and previous findings would suggest the direct involvement of DA, and/or its reactive metabolite(s), in the neurotoxicity of amphetamine-like compounds on the serotonergic system.

In summary, this report describes the neurochemical effects of MDE on the serotonergic and dopaminergic systems. These findings demonstrate that MDE has similar, although less potent, effects compared to those of MDA and MDMA on central serotonergic systems. The observation that the 5-HT system recovered at an earlier time from the MDE-mediated changes than those induced by MDA and MDMA treatments suggests that MDE is less neurotoxic than its analogues. The mechanisms of action for MDE or MDA and MDMA are unclear at this time. Due to the fact that all three compounds influence dopaminergic systems to some extent and that DA seems involved in the mechanism of the toxicity of amphetamines analogues on the serotonergic pathways, the role of DA in the toxic effects of MDA, MDMA and MDE needs to be examined further.

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* Dr. C. J. Schmidt, personal communication, cited with permission.

REFERENCES

1. A. T. Shulgin, in *Handbook of Psychopharmacology* (Eds. L. L. Iversen, S. D. Iversen and S. H. Snyder), Vol. 11, p. 243. Plenum Press, New York (1978).
2. D. E. Nichols, D. H. Lloyd, A. J. Hoffman, M. B. Nichols and G. K. W. Yim, *J. med. Chem.* **25**, 530 (1982).
3. C. J. Schmidt, J. A. Levin and W. Lovenberg, *Biochem. Pharmac.* **36**, 747 (1987).
4. D. M. Stone, D. C. Stahl, G. R. Hanson and J. W. Gibb, *Eur. J. Pharmac.* **128**, 41 (1986).
5. E. Sanders-Bush, J. A. Bushing and F. Sulser, *J. Pharmac. exp. Ther.* **192**, 33 (1975).
6. M. A. Peat, P. F. Warren, C. Bakhit and J. W. Gibb, *Eur. J. Pharmac.* **116**, 11 (1985).
7. U. Braun, A. T. Shulgin and G. Braun, *J. pharm. Sci.* **69**, 192 (1980).
8. P. C. Emson and O. Lindvall, *Neuroscience* **4**, 1 (1979).
9. T. Nagatsu, in *Methods in Biogenic Amine Research* (Eds. S. Parvez, T. Nagatsu, I. Nagatsu and H. Parvez), p. 329. Elsevier Science Publisher B.V., Amsterdam (1983).
10. A. Ichiyama, S. Nakamura, Y. Nishizuka and O. Hayaishi, *J. biol. Chem.* **245**, 1699 (1970).
11. B. R. Sitaram and G. J. Lees, *J. Neurochem.* **31**, 1021 (1978).
12. A. J. Hotchkiss, M. E. Morgan and J. W. Gibb, *Life Sci.* **25**, 1373 (1979).
13. J. A. Nielsen and K. E. Moore, *Pharmac. Biochem. Behav.* **16**, 131 (1982).
14. G. Ricaurte, G. Bryan, L. Strauss, L. Seiden and C. Schuster, *Science* **229**, 986 (1985).

15. D. M. Stone, M. Johnson, G. R. Hanson and J. W. Gibb, *Eur. J. Pharmac.* **134**, 245 (1987).
16. J. H. Biel and B. A. Bopp, in *Handbook of Psychopharmacology* (Eds. L. L. Iversen, S. D. Iversen and S. H. Snyder), Vol. 11, p. 1. Plenum Press, New York (1978).
17. S. Knapp, A. J. Mandell and M. A. Geyer, *J. Pharmac. exp. Ther.* **189**, 676 (1974).
18. G. A. Ricaurte, C. R. Schuster and L. S. Seiden, *Brain Res.* **193**, 153 (1980).
19. C. Bakhit, M. E. Morgan, M. A. Peat and J. W. Gibb, *Neuropharmacology* **20**, 1135 (1981).
20. J. A. Harvey, S. E. McMaster and L. M. Yunger, *Science* **187**, 841 (1975).
21. J. A. Harvey, *Ann. N.Y. Acad. Sci.* **305**, 289 (1978).
22. C. J. Schmidt and J. W. Gibb, *Neurochem. Res.* **10**, 637 (1985).
23. R. W. Fuller, K. W. Perry and B. B. Molloy, *J. Pharmac. exp. Ther.* **193**, 796 (1975).
24. C. J. Schmidt and W. Lovenberg, *Fedn Proc.* **45**, 5264 (1985).
25. R. W. Fuller and H. D. Snoddy, *J. Pharm. Pharmac.* **31**, 183 (1979).
26. S. B. Ross and A-L. Ask, *Acta pharmac. tox.* **46**, 270 (1980).
27. C. J. Schmidt and J. W. Gibb, *Eur. J. Pharmac.* **109**, 73 (1985).
28. E. M. Gál, P. A. Christiansen and L. M. Yunger, *Neuropharmacology* **14**, 31 (1975).
29. C. J. Schmidt, J. K. Ritter, P. K. Sonsalla, G. R. Hanson and J. W. Gibb, *J. Pharmac. exp. Ther.* **233**, 539 (1985).
30. A. J. Hotchkiss and J. W. Gibb, *J. Pharmac. exp. Ther.* **214**, 257 (1980).
31. L. S. Seiden and G. Vosmer, *Pharmac. Biochem. Behav.* **21**, 29 (1984).
32. H. Rollema, J. B. De Vries, B. H. C. Westerink, F. M. S. Van Putten and A. S. Horn, *Eur. J. Pharmac.* **132**, 65 (1986).
33. V. J. Massari, Y. Tizabi and D. Jacobowitz, *Eur. J. Pharmac.* **50**, 279 (1978).