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CHARACTERIZATION OF ACUTE *N*-ETHYL-3,4-METHYLENEDIOXYAMPHETAMINE (MDE) ACTION ON THE CENTRAL SEROTONERGIC SYSTEM

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Abstract—The effect of *N*-ethyl-3,4-methylenedioxyamphetamine (MDE) on the central serotonergic system was studied. Within 1 hr after administration of MDE (10 mg/kg), the concentration of 5-hydroxytryptamine (5-HT) and the activity of tryptophan hydroxylase (TPH) had declined significantly in the hippocampus but returned to control within 12 hr. Hippocampal 5-hydroxyindoleacetic acid (5-HIAA) content decreased within 2 hr, rebounded to 22% above control by 12 hr, and returned to control by 24 hr. Blockade of the 5-HT uptake carrier with fluoxetine (10 mg/kg) prevented or attenuated the MDE-induced changes in 5-HT content and TPH activity, except for neostriatal TPH activity which remained unresponsive to the fluoxetine treatment. The MDE-induced decline in TPH activity could be reversed by incubating the TPH preparation with dithiothreitol and Fe^{2+} under nitrogen for 24 hr. This suggests that the loss in TPH activity induced by MDE results from an alteration of the oxidation-reduction state of a sulfhydryl group located on the enzyme. The inhibition of monoamine oxidase (MAO) by the administration of pargyline (75 mg/kg) failed to protect the neostriatal TPH activity from the MDE-induced decline while potentiating the MDE-induced decrease in cortical TPH activity. This suggests that H_2O_2 generated by MAO *in vivo* is not responsible for oxidation of the sulfhydryl site located on TPH during the MDE treatment.

N-Ethyl-3,4-methylenedioxyamphetamine (MDE) is an amphetamine analogue sharing similar psychotomimetic activity with its analogue, 3,4-methylenedioxymethamphetamine (MDMA) [1]. The administration of a single dose of MDE causes a rapid decline in the concentration of central 5-hydroxytryptamine (5-HT) and in the activity of tryptophan hydroxylase (TPH), its biosynthetic enzyme [2, 3]. Similar effects on the serotonergic system have already been reported for several amphetamine analogues [4–6], including MDMA [7, 8]. However, in contrast to MDMA, a single dose of 40 mg/kg MDE fails to induce the long-term changes in the central serotonergic system associated with neuronal degeneration, although multiple doses of MDE do cause these neurotoxic effects [3, 9, 10]; thus, MDE has less neurotoxic potency. This property makes MDE a drug of choice to study the rapid but reversible decline in TPH activity following a single dose of amphetamine analogues.

The mechanism by which amphetamine analogues induce the rapid reversible decline in central TPH activity remains unclear. The recent observation made in our laboratory [11] indicates that the rapid inactivation in TPH activity induced by amphetamine analogues may be attributed to a change in the oxidation-reduction state of sulfhydryl groups in the enzyme structure. The activity of the enzyme can be restored by incubating under anaerobic conditions the TPH preparation with Fe^{2+} plus dithiothreitol (DTT), a potent reducing agent of disulfide bonds [12]; however, the depletion of TPH activity associated with the neuronal degeneration resulting from multiple administrations of MDMA cannot be reversed by this treatment [13]. The similarity between the rapid inactivation of TPH resulting from exposure to amphetamine analogues and by exposure to oxygen [14] suggests that amphetamine analogues, including MDE, favor the generation of an oxidant which inactivates the TPH enzyme.

The purpose of this study was to characterize further the rapid decline in central TPH activity following the acute administration of MDE, and to determine if monoamine oxidase (MAO) could be involved in the generation of an oxidant responsible for the decrease in TPH activity. MAO metabolizes dopamine and 5-HT which results in the generation of H_2O_2 [15], an oxidant suggested to inactivate TPH [16]. A large amount of H_2O_2 could be generated *in vivo* by MAO following the metabolism of drug-released dopamine and 5-HT. Thus, if H_2O_2 generated by MAO is involved in the acute decline in TPH activity induced by amphetamine analogues, the inhibition of MAO should protect TPH activity from the effects of an acute MDE administration.

METHODS

Drug administration. Five to six male Sprague–Dawley rats (180–250 g) were housed 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.* The animals were injected subcutaneously with (*dl*)-MDE hydrochloride (supplied by the National Institute on Drug Abuse) or 0.9% saline and killed 3 hr later, unless stated otherwise. The doses of MDE used in this study are expressed as the

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free base. Fluoxetine (10 mg/kg, i.p.; Lilly Research Laboratories, Indianapolis, IN) was administered 15 min prior to MDE administration, while pargyline (75 mg/kg, i.p.; Sabar Laboratories Inc., Morton Grove, IL) was administered 1 hr before the MDE treatment. The animals were killed by decapitation between 10:00 a.m. and 3:00 p.m. The brains were rapidly removed, and the frontal cortex (corresponding to the pregenual part of the anteromedial cortex described by Emson and Lindvall [17]), neostriatum and hippocampus were dissected out on a cold plate, frozen on dry ice, and stored at −80° until assayed.

Tryptophan hydroxylase (TPH) assay. Frontal cortex, hippocampus and neostriatum were homogenized in 80, 200 and 125 μl, respectively, of 5 mM DTT, 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (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 [18, 19] using (±)-6-methyl-5,6,7,8-tetrahydrobiopterin (Sigma Chemical Co., St Louis, MO) 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 for the TPH assay are described by Hotchkiss *et al.* [20].

Reconstitution of TPH activity. The conditions for this experiment are a modification of those described by Kuhn *et al.* [14]. Neostriatal tissue was homogenized and centrifuged as indicated for the TPH assay. Thirty microliters of the supernatant fraction was added into a 6 × 50-mm silanized glass tube containing 5 μl of a 5 mM DTT solution with ferrous ammonium sulfate to obtain a final Fe^{2+} concentration of 50 μM. The tubes were then placed in a Nalgene vacuum desiccator (without the desiccant) and gassed with nitrogen for 20 hr (at 24°). Following the anaerobic incubation, a 7.5-μl aliquot of the enzyme preparation was used to determine TPH activity. The enzyme activity was corrected to take into account the dilution resulting from addition of the DTT and Fe^{2+} solution.

Assay for 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). The determination of 5-HT and 5-HIAA concentrations using HPLC has been desribed elsewhere [2]. Briefly, tissues were weighed and 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 (v/v). The supernatant fraction resulting from a 30-min centrifugation (4000 *g* at 4°) was filtered with a 0.2-μm Microfilter system (Bioanalytical Systems Inc., West Lafayette, IN), and a 50-μl aliquot was injected onto a 12.5-cm Whatman C_{18} PartiSphere column (5 μm) connected to a reversed phase guard column (Whatman Inc., Clifton, NJ) using an autosampler (WISP model 710B, Waters, Milford, MA). The eluent was monitored with an amperometric detector (model LC-4B, Bioanalytical Systems Inc.) using a glassy carbon electrode with the potential set at +0.73 V (vs Ag/AgCl reference electrode).

Statistics. A one-way analysis of variance (ANOVA) followed with a Scheffe multiple comparison test was used to analyze the data presented in this study, except for Figs. 1 and 3 where MDE-treated animals were compared with their respective control with a Student's *t*-test.

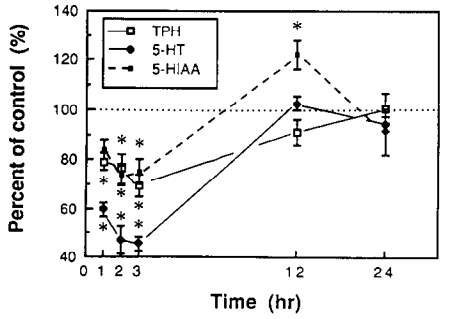

Fig. 1. Time-course of hippocampal TPH activity and of the concentration of 5-HT and 5-HIAA following administration of MDE. Rats were injected with either saline or MDE (10 mg/kg, s.c.) and killed at the times indicated. Means ± SE of the activity of TPH and of the concentration of 5-HT and 5-HIAA are expressed as percent of their respective time-matched control. Means ± SE of 5-HT concentration measured in the control group killed 1 hr after the administration of MDE was 0.37 ± 0.02 μg/g tissue while the value for the concentration of 5-HIAA was 0.25 ± 0.02. The activity of TPH measured in that control group was 60.3 ± 6 nmol of hydroxylated tryptophan/g tissue/hr. Statistical analyses were performed with a Student's *t*-test to compare the results from the MDE-treated groups with their respective control. Key: (*) $P < 0.05$ vs corresponding control (N = 5 or 6).

RESULTS

The time-course of the changes induced by a single dose of MDE (10 mg/kg) on hippocampal TPH activity as well as on hippocampal 5-HT and 5-HIAA concentrations is presented in Fig. 1. The activity of TPH and the concentration of 5-HT were depressed significantly to 79 and 60% of their respective time-matched controls within 1 hr following administration of MDE, whereas the decrease in concentration of 5-HIAA (84% of control) was significant only 2 hr after MDE administration. TPH activity was maximally depressed 3 hr after drug treatment, whereas the decrease in 5-HT and 5-HIAA concentrations was maximal after 2 hr. As previously reported [2], MDE induced a greater depletion in the concentration of 5-HT than in the activity of TPH. By 12 hr following MDE administration, the activity of TPH and the concentration of 5-HT returned to control levels, while the concentration of 5-HIAA was elevated significantly (22% of time-matched control). The concentration of 5-HIAA had returned to control by 24 hr.

The effects of the serotonergic uptake blocker, fluoxetine (10 mg/kg, i.p.), on the decreases induced by acute MDE treatment (10 mg/kg, s.c.) on central TPH activity, and on the concentrations of 5-HT and 5-HIAA are presented in Fig. 2. The ability of fluoxetine to block the MDE-induced changes varied according to the brain structure surveyed and on

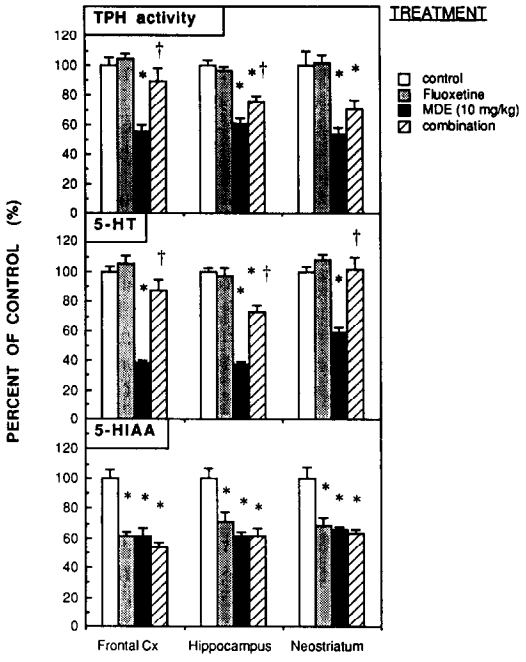

Fig. 2. Effect of fluoxetine treatment on MDE-induced changes. Rats were injected with fluoxetine (10 mg/kg, i.p.) 15 min prior to administration of saline or MDE (10 mg/kg, s.c.), and killed 3 hr later. TPH activities are expressed as percent (mean ± SE) of the control group (treated with saline alone) for the respective brain structures. Means ± SE of the TPH activities in the controls, expressed as nmol of hydroxylated tryptophan/g tissue/hr, were: 108.6 ± 5.6 in the frontal cortex; 76.5 ± 2.3 in the hippocampus; and 52.2 ± 5.1 in the neostriatum. Means ± SE of the 5-HT concentrations in the controls, expressed as μg/g tissue, were 0.55 ± 0.02 in the frontal cortex; 0.37 ± 0.01 in the hippocampus; and 0.53 ± 0.02 in the neostriatum. Means ± SE of the 5-HIAA concentrations in the controls, expressed as μg/g tissue, were: 0.13 ± 0.01 in the frontal cortex; 0.21 ± 0.02 in the hippocampus; and 0.38 ± 0.03 in the neostriatum. Statistical analyses were performed with a one-way ANOVA followed with a Scheffe multiple comparison test. Key: (*) $P < 0.05$ vs control, and (†) $P < 0.05$ vs MDE-treated group (N = 5 or 6).

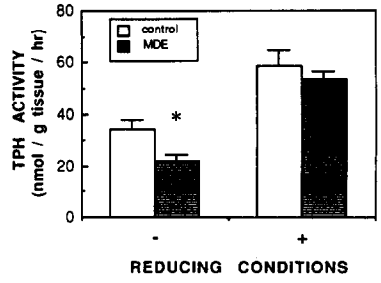

Fig. 3. Effect of anaerobic incubation under reducing conditions on the MDE-induced decline in neostriatal TPH activity. Rats were injected with MDE (20 mg/kg, s.c.) and killed 3 hr later. Neostriata were homogenized and centrifuged, and an aliquot from the supernatant fraction was assayed immediately for TPH activity (- reducing condition). Fe^{2+} was added to another aliquot and incubated for 24 hr under nitrogen atmosphere before being assayed for TPH (+ reducing conditions). TPH activities shown in this figure are expressed as nmol of hydroxylated tryptophan/g tissue/hr (mean ± SE). Statistical analyses were performed with a Student's *t*-test to compare the results from the MDE-treated group with their respective control. Key: (*) $P < 0.05$ (N = 5 or 6).

the serotonergic parameter measured. The uptake blocker completely prevented the MDE-induced decrease in cortical TPH activity while attenuating the drug-induced decrease in the hippocampus (Fig. 2, top panel). Fluoxetine failed to alter significantly the MDE-induced decline in neostriatal TPH activity. The serotonergic uptake blocker prevented the drug-induced decline in cortical and neostriatal 5-HT concentrations while attenuating the MDE response in the hippocampus (Fig. 2, center panel). Finally, fluoxetine alone depleted the 5-HIAA concentrations in the three brain structures surveyed to the level reached by MDE treatment (Fig. 2, bottom panel). The 5-HIAA concentration in rats receiving both MDE and fluoxetine remained depressed.

The results presented in Fig. 3 demonstrate that the depression in neostriatal TPH activity resulting from a single administration of MDE (20 mg/kg) was restored under reducing anaerobic conditions. MDE treatment reduced TPH activity to 64% of control. After anaerobic incubation under reducing conditions (5 mM DTT + 50 μM Fe^{2+} under nitrogen gas), TPH activity from the MDE-treated group was restored to the level of control. Failure to incubate under anaerobic conditions results in the loss of enzymatic activity [11].

To determine whether MAO activity is involved in the mechanism leading to the MDE-induced depletion of central TPH activity, different doses of MDE were administered 1 hr after rats received a 75 mg/kg dose of pargyline (Fig. 4). This treatment alone led to an 80% decline in the concentration of neostriatal 5-HIAA concentration (results not presented). As shown in Fig. 4, pargyline treatment significantly potentiated the effect of the 20 mg/kg dose of MDE on cortical TPH activity measured 3 hr after MDE administration. This potentiating action was also seen in the hippocampus (results not shown). The inhibition of MAO activity did not change the MDE effect measured in the neostriatum.

DISCUSSION

The results presented in this study demonstrate that a single administration of MDE produces a profound but short-lived decline in the concentrations of 5-HT and 5-HIAA and the activity of TPH. These changes can be attentuated or prevented by the administration of a serotonergic uptake blocker. The MDE-induced decrease in TPH activity results most likely from a change in the oxidation state of sulfhydryl groups located on the enzyme. This drug-induced change is apparently not linked to the generation of H_2O_2 from MAO as inhibition of this enzyme failed to block the MDE effect on central TPH activity.

MDE is like MDMA in producing a rapid decline in 5-HT and 5-HIAA [7, 8, 21, 22] but unlike

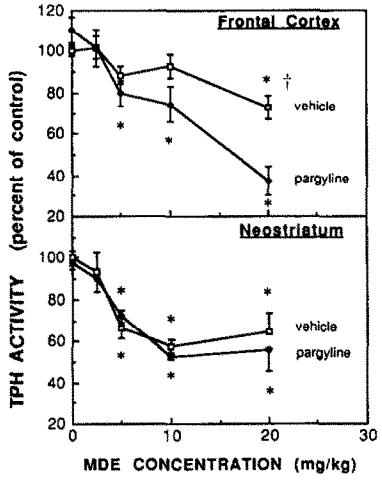

Fig. 4. Effect of MAO inhibition on the MDE-induced decrease in central TPH activity. Rats were injected with pargyline (75 mg/kg, i.p.) or saline 1 hr prior to the administration of different doses of MDE (2.5 to 20 mg/kg, s.c.) or saline, and killed 3 hr after the last injection. Means of TPH activities are expressed as percent of control (0 mg/kg MDE on vehicle curve) ±SE. Means ± SE of the TPH activities in the controls, expressed as nmol of hydroxylated tryptophan/g tissue/hr, were 61.8 ± 1.6 (N = 18) in the frontal cortex; and 45.5 ± 1.6 (N = 17) in the neostriatum. Statistical analyses were performed with a one-way ANOVA followed with a Scheffe multiple comparison test. Key: (*) $P < 0.05$ vs 0 mg/kg MDE, and (†) $P < 0.05$ vs respective pargyline-treated group (N = 5–7).

MDMA in that the changes were transient with MDE (Fig. 1) but long-lasting with MDMA. This acute depletion of 5-HT and 5-HIAA concentrations following MDE administration can be explained by the drug-induced release of the transmitter [3] and by the decrease in its rate of synthesis (Fig. 1). However, unlike MDMA, MDE fails to alter the 5-HT concentration measured 7 days after a single dose of the drug [3]. This discrepancy can be explained by the lower potency of MDE to induce neuronal degeneration which accounts for the long-term decline in 5-HT concentration [7, 8]. These observations indicate that the effects of MDE and MDMA on the serotonergic system are similar shortly after the drug administration, but differ after a certain period of time. As described for 5-HT, the MDE-induced decrease in TPH activity was also temporary, thus contrasting with the long-lasting decrease in TPH observed after MDMA treating which is likely due to the gradual development of neuronal degeneration in the serotonergic system [7, 8].

Possible explanations for the rapid loss in central TPH activity induced by MDE and other amphetamine analogues are: (1) an increase in the catabolism of TPH or a loss in the integrity of serotonergic terminals, (2) the direct inhibition of the enzyme with amphetamine analogues, or (3) a reversible change in the structure of the enzyme. The first explanation is unlikely in light of the results presented in Figs. 1 and 3. The rapid reconstitution of TPH activity and the return of 5-HT concentrations within 12 hr of drug administration suggest that the serotonergic terminals maintained their structural integrity after MDE treatment. Moreover, the restoration of TPH activity *in vitro* by anaerobic incubation suggests that TPH remains in the nerve terminal but is inactive after acute MDE treatment rather than being eliminated through a catabolic cycle. The direct inhibition of TPH by amphetamine analogues is also an unlikely explanation considering that *in vitro* studies have demonstrated that these drugs are poor enzyme inhibitors [4, 8]. Thus, the demonstration that DTT can restore the TPH activity lost shortly after the administration of MDE (Fig. 3), or after other amphetamine analogues [11], suggest that a more likely mechanism is a change in the enzyme structure leading to inactivation of TPH.

The recovery of TPH activity induced by DTT and Fe^{2+} under anaerobic conditions supports our previous observations with MDMA [11]. We demonstrated that DTT is required to restore the decreased enzymatic activity 3 hr after acute treatment with MDMA. Because DTT can break disulfide bonds as a result of its potent reducing action on thiol groups [12], it is suggested that the reduction of sulfhydryl sites on TPH is responsible for this recovery in enzymatic activity. On the other hand, this suggests that the MDMA- and the MDE-induced decline in TPH activity measured shortly after the drug administration may result from an alteration of a sulfhydryl site. Interestingly, anaerobic incubation with DTT failed to restore the loss of TPH activity measured 7 days after a single dose of MDMA [11]. This provides further support that the rapid loss in TPH activity induced by MDMA can be reversed *in vitro*, as observed with MDE, while the long-term decrease resulting from neuronal degeneration is not. Because of its lower potency to induce neuronal degeneration, a single administration of 10 mg/kg MDE does not cause the long-term changes in the serotonergic system [3].

The exact nature of the rapid transformation of TPH into an inactive form following MDE treatment is still uncertain; however, the formation of a disulfide bound within the active site of TPH with a consequent loss of activity is possible. The binding of a substance generated by MDE treatment to the enzyme is also possible; recently, Ito and collaborators [23] described the binding of *o*-quinone products generated from catecholamines on a sulfhydryl site located on albumin. The generation of such an *o*-quinone would be consistent with the observation that dopamine is essential for decreases in TPH activity induced by methamphetamine [24, 25]. Drug-released dopamine could enter the serotonergic terminals by the serotonergic uptake carrier, as already reported [26], and be transformed into a reactive metabolite which binds to a sulfhydryl site on the enzyme. However, the inability of DTT to reverse such binding [27] makes this model less attractive.

The loss of TPH activity after exposure to molecular oxygen is currently the most attractive model to explain the amphetamine inactivation. This inactivation can be reversed by incubating the enzyme preparation with DTT and Fe^{2+} under anaerobic conditions [14]. It is possible that treatment with MDE and other amphetamine analogues generates

an oxidant, or alters the oxidation-reduction state within the serotonergic terminals, which could impair TPH activity. In this study, we examined the possible role of MAO in generating such an oxidant. Amphetamine analogues induce a massive release of monoamines [3, 28] which are substrates of MAO [29]. The catabolism of these monoamines by MAO produces H_2O_2 [15]. Friedman *et al.* [16] suggested that TPH activity was sensitive to H_2O_2, while Hosoda [30] reported that TPH from malignant murine mast cells becomes more sensitive to H_2O_2 during purification.

The results presented in this study do not support involvement of MAO in the acute decline in TPH activity induced by amphetamine analogues. The inhibition of MAO activity with pargyline failed to protect TPH from the inactivation induced by the MDE treatment; indeed, the response to MDE was exacerbated in the frontal cortex of pargyline-treated animals. Thus, the activity of MAO and the generation of H_2O_2 by this enzyme do not appear to contribute to the drug-induced loss in TPH activity. It is interesting to note, however, that inhibition of MAO potentiated the effects of MDE in the frontal cortex but not in the neostriatum. The explanation for this difference is not yet apparent. One possible explanation derives from the fact that MAO inhibition increases tissue content of monoamines [31, 32] as well as increases the drug-induced release of these transmitters [33]. If dopamine mediates the MDE-induced decrease of TPH activity, as suggested for methamphetamine and MDMA [25, 34], the additional dopamine released by MDE after MAO inhibition could induce a greater decrease in cortical TPH activity. In the neostriatum, however, there is a greater concentration of dopamine available for release. MDE treatment alone may release enough neostriatal dopamine to produce its maximum effect on TPH; thus, a greater amount of dopamine released resulting from the MAO inhibition would not add to the response. Dopamine may not be the only factor mediating the MDE-induced decline in central TPH activity; inhibition of MAO, which would make more dopamine available for release, fails to potentiate the effect of low doses of MDE.

The ability of fluoxetine, an inhibitor of the 5-HT uptake carrier, to prevent the MDE-induced decline in TPH activity and in the concentration of 5-HT demonstrates that MDE has characteristics similar to those of other amphetamine analogues [3, 6, 35]. It is interesting to note that fluoxetine attenuated or blocked to a greater extent the MDE-induced decline in 5-HT content than the changes in TPH activity. This supports the observation made by Schmidt and Taylor [8] that citalopram, another 5-HT uptake carrier inhibitor, blocks the MDMA-induced decline in the concentration of cortical 5-HT measured 3 hr after administration of MDMA, while only attenuating the changes observed in TPH activity. This could be explained by the observation that 5-HT uptake blockers reduce the 5-HT release induced by amphetamine analogues [35], thus allowing the remaining active TPH to maintain the concentration in the intracellular 5-HT pool despite some loss in TPH activity. In our experiments, fluoxetine treatment also totally blocked the MDE-induced decrease in cortical TPH activity but did not alter significantly the response of the neostriatal enzyme. The explanation for this observation needs further investigation.

In summary, MDE produced a short-lasting decrease in the concentration of central 5-HT and 5-HIAA as well as in the activity of TPH. These immediate changes are characteristic of many amphetamine-like compounds, and can be prevented by inhibiting the 5-HT uptake carrier. In contrast with its analogue, MDMA, MDE induced changes in TPH activity that last only 12 hr. The rapid drug-induced decline in central TPH activity may result from a change in the oxidation state in sulfhydryl groups on the enzyme. This MDE effect appears similar to the loss in TPH activity which occurs after exposure to molecular oxygen. Thus, amphetamine analogues could facilitate the generation of an oxidant responsible for the inactivation of TPH. From experiments conducted in this study, H_2O_2 generated by MAO is apparently not the oxidant responsible for the loss in TPH activity.

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