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**Short communication**

# Reversal of the acute effects of 3,4-methylenedioxymethamphetamine by 5-HT uptake inhibitors

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Recent evidence suggests that the acute 3,4-methylenedioxymethamphetamine (MDMA)-induced loss of tryptophan hydroxylase activity (TPH) may be due to the oxidation of critical sulf-hydryl groups on the molecule. To determine if TPH activity could be regenerated in vivo we administered a 5-HT uptake inhibitor at various times immediately after MDMA. Although enzyme activity began to decline immediately following MDMA administration, rats receiving the uptake inhibitor 1 h post MDMA showed a rapid recovery of TPH activity. Administration of an uptake inhibitor 3 h post MDMA was without effect on the time course of TPH inactivation. The results suggest that systems exist within the serotonergic neuron for the reductive regeneration of active TPH. Furthermore, these systems are acutely compromised following the administration of MDMA.

Tryptophan hydroxylase; MDMA (3,4-methylenedioxymethamphetamine); 5-HT uptake inhibitors

## 1. Introduction

The unique behavioral effects of the amphetamine analog, 3,4-methylenedioxymethamphetamine (MDMA) have lead to its widespread abuse. Users report a heightened sense of self-awareness and an increased empathy without overt hallucination or sensory disturbances. Although these effects made MDMA a potential candidate for use as a psychoadjuvant, neurochemical studies revealed that MDMA and several of its congeners could produce serotonergic terminal degeneration in laboratory animals. Concerns over neurotoxicity and the high abuse liability of MDMA lead to its placement in Schedule 1 by the DEA.

In addition to the delayed degeneration of serotonergic nerve terminals, MDMA has more immediate neurochemical effects on serotonergic neurons. An acute, reversible decrease in brain concentrations of 5-HT occurs due to a rapid inactivation of tryptophan hydroxylase (TPH) coupled with the carrier-mediated efflux of available 5-HT from the nerve terminal. This loss of TPH activity can be observed within 15 min of drug administration and is due to an apparent inactivation of enzyme rather than a change in substrate affinities (Schmidt and Taylor, 1987; 1988). Similar acute losses of TPH activity have been demonstrated after the administration of methamphetamine or p-chloroamphetamine (PCA).

Recently, Stone et al. (1989) demonstrated regeneration of TPH activity from animals treated acutely with MDMA, methamphetamine or PCA by incubation of cortical homogenates under $N_2$ with reducing agents such as diethiothreitol or 2-mercaptoethanol. Their results suggested that MDMA administration leads to the acute oxidation of one or more critical sulfhydryl groups on the enzyme. We therefore questioned whether the

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oxidation of TPH was an irreversible step or if endogenous systems could also regenerate enzyme activity in vivo. Although a number of pharmacological treatments have been shown to interfere with the long-term or neurotoxic effects of MDMA, only inhibitors of the 5-HT uptake system have consistently been found to block the acute alterations in both 5-HT concentrations and TPH activity produced by the drug (Schmidt, 1987). We therefore administered 5-HT uptake inhibitors at several times immediately after MDMA to examine how inhibition of the carrier would affect the time course of 5-HT depletion and the loss of TPH activity.

## 2. Materials and methods

### *2.1. Drug administration*

Male Sprague-Dawley rats (250-300 g) were maintained on a 12 h light-dark cycle with free access to food and water. Fluoxetine and MDMA (as the free base) were administered s.c. in saline. The selective 5-HT uptake inhibitor, MDL 27,777 (Freedman et al., 1989), was substituted for fluoxetine in some experiments due to its greater availability. Animals were routinely dosed between 9:00-11:00 h and killed in the afternoon. Rats were killed by decapitation, the brains were removed, rapidly dissected on ice and stored at −70 °C until assayed.

MDMA was provided by the National Institute on Drug Abuse (NIDA). Fluoxetine was the generous gift of Lilly Research Laboratories, (Indianapolis, IN), MDL 27,777 (2,3-dihydro-N-methyl-[4-(trifluoromethyl)-phenoxy]-1H-indene-2-methanamine) was synthesized at the Merrell Dow Research Institute by Dr. Jules Freedman.

### *2.2. Determination of tryptophan hydroxylase activity and 5-HT concentrations*

TPH activity was assayed by the coupled $^{14}CO_2$ trapping method described previously (Hotchkiss et al., 1979; Schmidt and Taylor, 1987) using aromatic L-amino acid decarboxylase partially purified from bovine kidney (Lovenberg and Engelman, 1971). Brain concentrations of 5-HT, dopamine and their metabolites were determined by high performance liquid chromatography with electrochemical detection as described previously (Schmidt and Taylor, 1988).

## 3. Results

Initial studies were conducted with the 5-HT uptake inhibitor fluoxetine and 20 mg/kg MDMA. Figure 1 provides data on cortical TPH activity and 5-HT concentrations from an experiment in which fluoxetine (5 mg/kg) was administered 3 h after MDMA. As illustrated by this figure, the loss of enzyme activity appears to plateau between 3

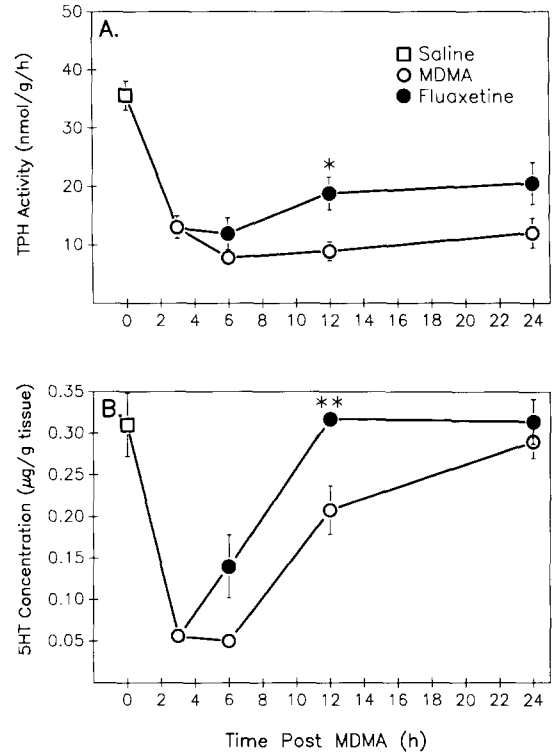


Fig. 1. Failure to reverse the acute effects of MDMA by administration of a serotonin uptake inhibitor (fluoxetine, 5 mg/kg s.c.) 3 h post MDMA (20 mg/kg s.c.). Results shown are for cortical TPH activity (A) and cortical 5-HT concentrations (B). Each point is the mean ± S.E.M. for five animals. **$P < 0.01$ compared to MDMA by the two-tailed Student's t-test.

and 6 h with no recovery out to 24 h. When administered 3 h post MDMA, fluoxetine had minimal effect on the loss of TPH activity. Cortical 5-HT concentration showed a slightly accelerated rate of recovery in animals treated with the uptake inhibitor. Similar results were observed in the striatum (data not shown).

In the next series of experiments, the selective 5-HT uptake inhibitor MDL 27,777 (5 mg/kg s.c.) was administered either 1 or 2 h post MDMA. The dose of MDMA was also reduced to 10 mg/kg due to the shorter duration of these experiments. Previous work demonstrated no significant difference in the acute effects of 10 or 20 mg/kg MDMA (Schmidt and Taylor, 1987). The effects of MDL 27,777 on cortical TPH activity and 5-HT concentrations following MDMA administration are shown in fig. 2. The rapid decline in cortical enzyme activity was halted and actually reversed by the uptake inhibitor. Although TPH activity was only monitored out to 6 h post MDMA, a statistically significant increase in enzyme activity could be demonstrated between 1 and 6 h after MDMA. Evidence of a slower recovery of activity was observed in the 2 h post MDMA treatment group although the changes between 3 and 6 h were not statistically significant. The rapid depletion of cortical 5-HT was also reversed by the uptake inhibitor. Values near control were reestablished within 4-5 h of administration of MDL 27,777 in spite of depletions of greater than 50% at the time the drug was given. Identical results were observed for both hippocampal TPH activity and 5-HT concentrations. The results with MDL 27,777 were verified in a similar experiment in which fluoxetine (5 mg/kg) was administered 1 h after MDMA. As observed with MDL 27,777, indices of serotonergic function showed a rapid recovery following administration of the uptake inhibitor (data not shown).

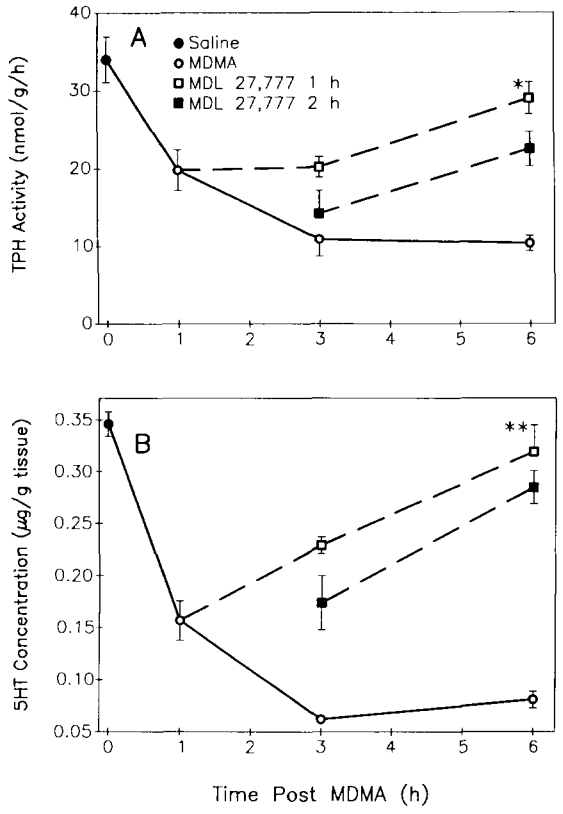


Fig. 2. Reversal of the acute effects of MDMA by administration of a 5-HT uptake inhibitor (MDL 27,777, 5 mg/kg s.c.) either 1 or 2 h post MDMA (10 mg/kg). Results shown are for cortical TPH activity (A) and cortical 5-HT concentrations (B). Each point is the mean ± S.E.M. for five animals. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to MDMA at 1 h by the two-tailed Student's t-test.

## 4. Discussion

The observation by Stone et al. (1989), that TPH inactivated by MDMA and related agents in vivo could be regenerated by reducing agents in vitro demonstrated that amphetamines may be affecting the redox state of the serotonergic nerve terminal. One early sign of this imbalance may be the loss of TPH activity. TPH is an extremely labile enzyme largely due to the sensitivity of its sulfhydryl groups to molecular oxygen (Kuhn et al., 1980). It therefore seems reasonable that the serotonergic neuron must possess some means of maintaining the enzyme in its reduced and hence active form. Although rapid, the loss of TPH activity after MDMA administration is a cumulative process that may represent the consumption of the substance(s) responsible for maintaining active TPH. We reasoned that interrupting the process leading to the loss of enzyme activity at an early time point might allow the endogenous regeneration of the enzyme. Although the mecha-

nism is not understood, inhibitors of the 5-HT uptake carrier have been found to protect against the acute loss of TPH activity and the subsequent depletion of 5-HT concentration. Our results indicate that administration of the uptake inhibitor, while enzyme activity is still declining, can reverse the loss of TPH activity. In our hands, this is 1-2 h after the administration of MDMA. By 3 h we were unable to demonstrate any recovery of enzyme activity even out to 24 h. It is interesting that the point at which the system responsible for keeping TPH in the reduced form is finally exhausted is also the point of maximum loss of enzyme activity. Since full recovery of enzyme activity was possible at 3 h in the in vitro studies of Stone et al. (1989) these results suggest that depletion of the endogenous reductants is responsible for the failure to demonstrate endogenous reactivation of TPH rather than actual destruction of the enzyme at this time point.

These early effects of MDMA and related agents may play a significant role in the subsequent neurotoxicity produced by these drugs. The inability of the serotonergic neuron to maintain TPH activity following MDMA administration suggests that the terminal may be extremely vulnerable to any subsequent metabolic perturbation, particularly oxidative insult. Previous work form this laboratory has demonstrated that the administration of fluoxetine as long as 6 h after MDMA can still significantly attenuate the serotonergic deficits measured 1 week later (Schmidt, 1987). Since the acute effects of MDMA are fully developed by 6 h, these results suggest that a second event involving the 5-HT uptake carrier is necessary for the commitment to terminal degeneration. If this second event is also oxidative in nature, the already compromised serotonergic terminal may be permanently damaged by the event. The acute effect of MDMA would therefore be a prerequisite, but not irreversible, step towards neurotoxicity.

The role of early oxidative stress in the eventual neurotoxicity of MDMA and its analogs may to some extent explain the selectivity of these neurotoxins for nerve terminals. We have previously demonstrated that the acute loss of TPH activity in the terminal fields of the serotonergic system is not observed in the cell body region following MDMA (Schmidt and Taylor, 1988). Given the greater size and metabolic reserves of a cell body in comparison to the nerve terminals, one explanation for this difference would be that the cell bodies have a greater capacity to deal with oxidative stress. Thus the failure of MDMA to affect TPH activity in the cell bodies may also be the basis of their resistance to subsequent irreversible damage.

In conclusion, the acute oxidative inactivation of TPH following MDMA administration can be reversed by early administration of inhibitors of the 5-HT uptake system. The failure of such treatments to regenerate TPH activity by 3 h post MDMA indicates that the endogenous systems responsible for the reactivation are depleted by this time point. Since these system(s) are likely to be antioxidant in nature, their depletion may be a predisposing factor in the neurotoxicity of MDMA.

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