Methylenedioxymethamphetamine-induced hyperthermia and neurotoxicity are independently mediated by 5-HT_2 receptors

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Methylenedioxymethamphetamine (MDMA) produced a significant hyperthermia in rats which was antagonized in a competitive manner by the selective 5-HT_2 antagonist, MDL 11,939. The 5-HT antagonist also blocked MDMA-induced neurotoxicity as assessed by the decline in regional 5-HT concentrations observed 1 week later. These two effects of MDL 11,939 were dissociated at higher doses of MDMA where the antagonist still provided virtually complete protection against the neurochemical deficits but only partially attenuated the hyperthermic response. In contrast to the effect of the 5-HT_2 antagonist, haloperidol did not alter MDMA-induced hyperthermia but did antagonize its long-term neurochemical effects. Similarly, coadministration of the selective 5-HT uptake inhibitor, MDL 27,777, did not affect the hyperthermia produced by a high dose of MDMA but completely prevented the depletion of 5-HT. When the MDMA-induced hyperthermia was prevented by temporarily maintaining animals at reduced ambient temperature, the neurochemical changes normally observed 1 week later were also blocked. Although these results demonstrate that the drugs tested do not antagonize MDMA-induced neurotoxicity by interfering with its effect on body temperature, they do indicate that MDMA-induced hyperthermia may contribute to the development of the drug's long-term neurochemical effects.

INTRODUCTION

The administration of a single high dose of methylenedioxymethamphetamine (MDMA) to rats produces an immediate, reversible decrease in brain 5-HT concentrations. This is followed by a second and more persistent depletion several days later[14]. Both biochemical[14] and immunohistochemical[10] studies have correlated this later phase of 5-HT depletion with a degeneration of serotonergic nerve terminals similar to that seen following the administration of *p*-chloroamphetamine[9]. The recreational abuse of MDMA has provoked considerable interest in this neurotoxicity with regard to both its possible mechanism and the functional consequences of such serotonergic deficits.

Attempts to elucidate the mechanism of MDMA-induced neurotoxicity have indicated dopamine release is a required event in the process[16,18]. The long-term or neurotoxic effects produced by high doses of amphetamine or methamphetamine show a similar dependence on intact dopamine stores[4,6,13]. This common feature suggests that the neurotoxic amphetamines may share a common mechanism of action. An unexpected observation in our studies of MDMA-induced neurotoxicity was the protection provided by selective 5-HT_2 antagonists[16]. As these results were not entirely consistent with a dopamine-based mechanism of neurotoxicity, the possibility of non-specific antagonism was considered. It is well documented that 5-HT receptors play a role in temperature regulation and the hyperthermia produced by the amphetamines[2]. Furthermore, a protective effect of hypothermia on excitatory amino acid-induced neurotoxicity has recently been described[1]. The studies reported here were performed to determine what role MDMA-induced hyperthermia might play in the long-term neurochemical deficits produced by MDMA and if the protection provided by 5-HT_2 antagonists could be attributed to a blockade of this effect.

MATERIALS AND METHODS

All experiments were performed using male Sprague–Dawley rats (250–300 g) housed on a 12-h dark–light cycle and given food and water ad libitum. MDMA and the antagonists were administered at 1 ml/kg in saline by subcutaneous injection. MDL 11,939 and MDL 27,777 were synthesized at the Merrell Dow Research Institute (Cincinnati, OH). Haloperidol was purchased from Research Biochemicals (Natick, MA). All doses were calculated for the salt form of the drug except MDMA which was given as the free base. MDMA·HCl was generously provided by the National Institute on Drug Abuse (NIDA).

Ambient temperature was recorded prior to all experiments and was generally in the range of 24–26 °C. A cold room was adjusted to 10 °C for experiments at reduced ambient temperature. Rectal temperatures were measured using a BAT 10 Thermometer with

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either a RET 1 or RET 2 thermocouple probe (Sensortek, Clifton, NJ). The probe was inserted 2 cm into the rectum and maintained until the temprature reading stabilized.

For neurochemical studies, rats were killed 1 week after the temperature measurements by decapitation. Brain regions were dissected on ice and tissues were stored at −80 °C until assayed. Regional 5-HT concentrations were measured by high-performance liquid chromatography (HPLC) with electrochemical detection as described previously[14].

Neurochemical results were analyzed for significance using one-way analysis of variance and the Student–Newman–Keuls test. Body temperature measurements were compared using a two-tailed Student's *t*-test. Significant differences for both tests were set at $P < 0.05$.

RESULTS

Effect of receptor antagonists on hyperthermia and neurochemistry

The administration of 10–30 mg/kg MDMA produced a rapid rise in body temperature which reached its maximum at approximately 60 min and remained elevated for the 3 h duration of the experiment ($P < 0.001$). Fig. 1A–C demonstrates the antagonism provided by coadministration of the selective 5-HT_2 receptor antagonist, MDL 11,939[3] (5 mg/kg, s.c.), at each dose of MDMA. At 10 mg/kg MDMA, administration of the antagonist completely blocked the hyperthermic effect. Although 20 mg/kg of MDMA produced a quantitatively similar increase in body temperature, MDL 11,939 partially but significantly antagonized the effect initially ($P < 0.05$) but appeared to totally block the hyperthermia at 3 h ($P < 0.01$). At 30 mg/kg MDMA, a significant hyperthermia was observed at all time points in the presence of the antagonist although a statistically significant attenuation was observed at 2 and 3 h ($P < 0.05$). In contrast to the effect of the 5-HT_2 antagonist, a high dose of the dopamine receptor antagonist, haloperidol (2 mg/kg, i.p.), had no effect on the hyperthermia produced by 20 mg/kg MDMA at any of the time points examined (data not shown).

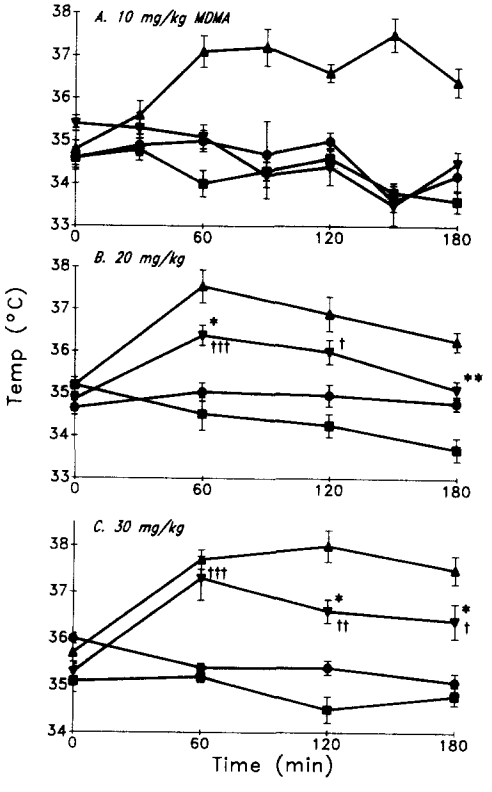

Fig. 1. Antagonism of the hyperthermia produced by various doses of MDMA in the rat. Complete blockade of MDMA-induced hyperthermia was observed only at the lowest dose of MDMA (A). Each point is the mean ± S.E.M. for 5 or more animals. The hyperthermia produced by MDMA was significant at all points shown ($P < 0.01$). ●——●, saline; ▲——▲, MDMA; ■——■, MDL 11,939; ▼——▼, MDMA + MDL 11,939. $^{*}P < 0.05$, $^{**}P < 0.01$ vs MDMA alone, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs saline.

Rats administered 20 or 30 mg/kg MDMA were sacrificed 1 week later to determine the effect of the receptor antagonists on MDMA-induced neurotoxicity. Fig. 2A–C displays the results of the 20 mg/kg experiments for cortical, hippocampal and striatal 5-HT concentrations, respectively. Both haloperidol and MDL 11,939 attenuated the long-term depletion of 5-HT concentrations produced by MDMA to a similar extent in all 3 regions ($P < 0.05$). The results for the 30 mg/kg MDMA group are shown in Fig. 3A–C. Although unable to block the hyperthermia produced by the high dose of MDMA, the antagonist still provided virtually complete protection against the serotonergic deficits in all 3 regions

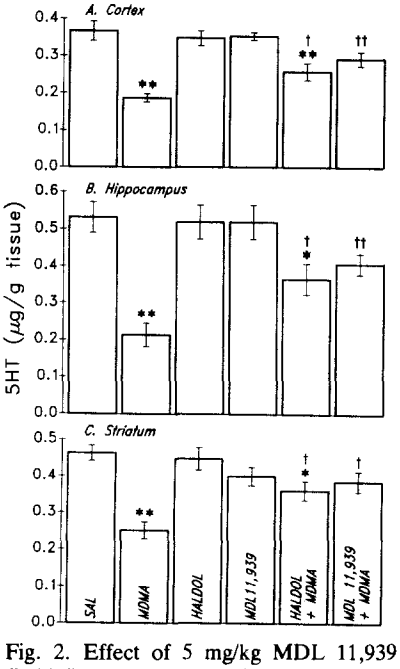

Fig. 2. Effect of 5 mg/kg MDL 11,939 or 2 mg/kg haloperidol (haldol) on the decrease in regional 5-HT concentrations produced 1 week after the acute administration of 20 mg/kg MDMA. $^{*}P < 0.05$, $^{**}P < 0.01$ vs saline; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs MDMA.

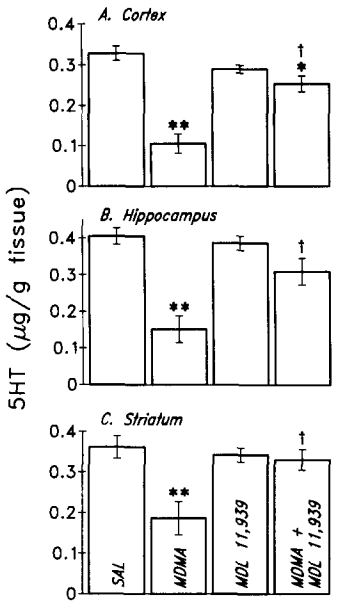

Fig. 3. Effect of MDL 11,939 (5 mg/kg) on the decrease in regional 5-HT concentrations produced 1 week after the acute administration of 30 mg/kg MDMA. $^{*}P < 0.05$, $^{**}P < 0.01$ vs saline; $^{\dagger}P < 0.05$ vs MDMA.

($P < 0.05$). None of the treatments modified striatal dopamine or its metabolites (data not shown).

Effect of inhibition of the 5-HT uptake carrier on the hyperthermic and neurotoxic response to MDMA

MDMA-induced neurotoxicity has been demonstrated to be sensitive to inhibitors of the 5-HT uptake carrier. Coadministration of the 5-HT uptake inhibitor MDL

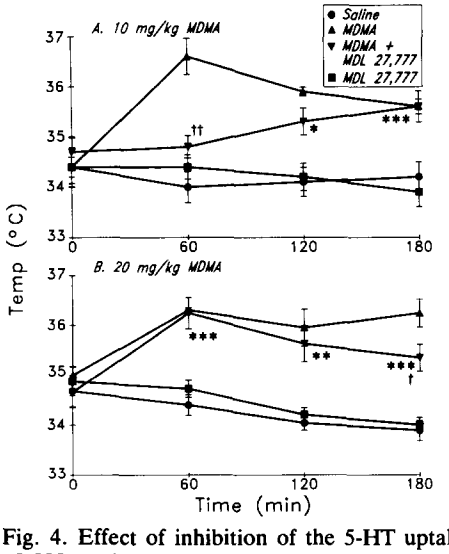

Fig. 4. Effect of inhibition of the 5-HT uptake carrier with MDL 27,777 on the hyperthermia produced by 10 mg/kg (A) or 20 mg/kg (B) MDMA. Each point is the mean ± S.E.M. for 5 or more animals. The hyperthermia produced by MDMA was significant at all points shown ($P < 0.001$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs saline; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs MDMA.

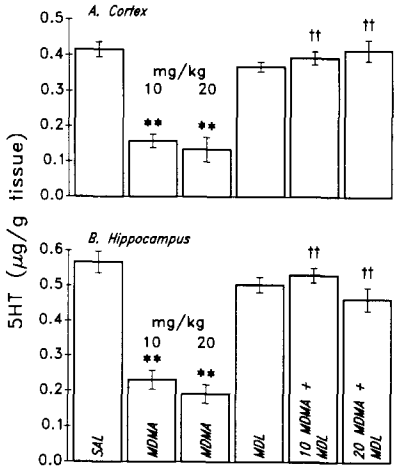

Fig. 5. Blockade of the long-term depletion of cortical (A) and hippocampal (B) 5-HT concentration produced by 10 mg/kg or 20 mg/kg MDMA by MDL 27,777 (5 mg/kg). $^{**}P < 0.01$ vs saline; $^{\dagger\dagger}P < 0.01$ vs MDMA.

27,777 (5 mg/kg) with 10 mg/kg MDMA also antagonized MDMA-induced hyperthermia ($P < 0.01$) but only at the earliest time point (Fig. 4A). By 3 h the MDL 27,777 plus MDMA group was indistinguishable from MDMA alone. When the dose of MDMA was increased to 20 mg/kg the effect of the uptake inhibitor observed after 10 mg/kg MDMA was abolished (Fig. 4B).

The neurochemical results from the same experiments are shown in Fig. 5. MDL 27,777 completely blocked the reduction in cortical and hippocampal 5-HT produced by either dose of MDMA ($P < 0.01$).

Effect of ambient temperature on MDMA-induced hyperthermia and neurotoxicity

To determine if the hyperthermia produced by MDMA was important in the long-term neurochemical effects of the drug, rats were administered MDMA (20 mg/kg) at

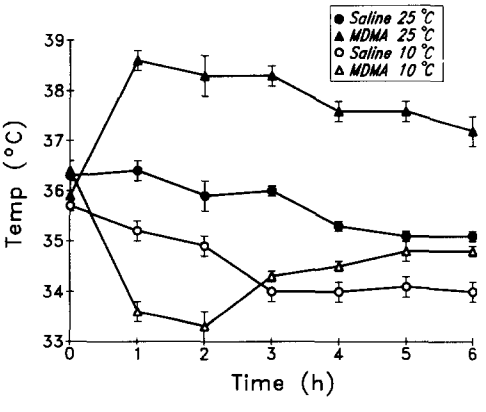

Fig. 6. Blockade of MDMA-induced hyperthermia at a reduced ambient temperature. The hyperthermia produced by MDMA was significant at all time points shown ($P < 0.001$). Each point is the mean ± S.E.M. for 5–6 animals.

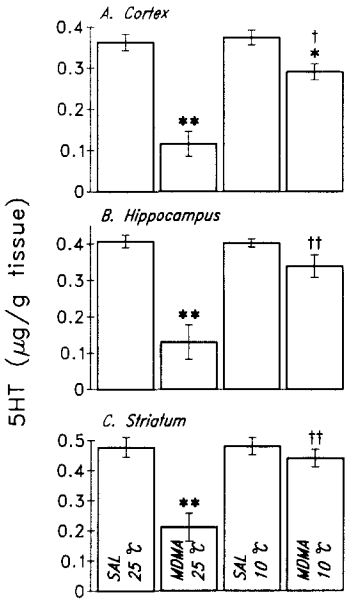

Fig. 7. Effect of interference with MDMA-induced hyperthermia (see Fig. 6) on the subsequent serotonergic deficits measured in cortex (A), hippocampus (B) and striatum (C) 1 week later. $^{*}P < 0.05$, $^{**}P < 0.01$ vs saline; $^{\dagger\dagger}P < 0.01$ vs MDMA.

room temperature (24–26 °C) or at 10 °C. Animals were maintained at the reduced temperature for 6 h then returned to room temperature facilities. As shown in Fig. 6, the lower ambient temperature not only blocked the development of MDMA-induced hyperthermia but in several of the experiments, converted the response to a hypothermia.

The long-term effects of MDMA on 5-HT concentrations in the cortex, hippocampus and striatum for these two groups of animals are shown in Fig. 7A, B and C, respectively. MDMA produced a large decrease in the concentration of 5-HT in all 3 regions when the animals were maintained at room temperature ($P < 0.01$). Maintaining the animals at a reduced ambient temperature significantly attenuated this effect in each region. Similar results were observed in two additional experiments.

An identical experiment was performed on rats sacrificed at 3 h to determine whether MDMA-induced hyperthermia was involved in the acute depletion of 5-HT produced by the drug. Although the hyperthermic response to MDMA (10 mg/kg) was again reversed by maintaining the rats at the reduced ambient temperature, the depletion of cortical and hippocampal 5-HT was identical to that measured in rats at room temperature (not shown).

DISCUSSION

The purpose of this study was to characterize the hyperthermia produced in rats by MDMA and determine whether interference with this activity could explain the protective effect of a diverse number of pharmacological agents for MDMA-induced neurotoxicity. Several recent studies have focused on the influence of brain temperature on the extent of neuronal degeneration following a metabolic insult such as cerebral ischemia. Reductions in brain temperature of only a few degrees have been found to prevent the appearance of neuronal damage in an occlusion model of ischemia[1]. If a hyperthermic response was necessary for some of the neurochemical effects of MDMA, blockade of this response by a 5-HT_2 antagonist could interfere with MDMA-induced neurotoxicity through an indirect manner.

There is a large body of literature dealing with the effects of direct and indirect serotonergic agonists on body temperature (for a review see Clark and Lipton[2]). In general, the ability of an indirect agonist, such as *p*-chloroamphetamine, to produce hypothermia or hyperthermia is dependent upon the ambient temperature[7,8]. To determine if the hyperthermia produced by MDMA was required for the development of the neurotoxic response, rats were maintained at a low ambient temperature for 6 h following the administration of MDMA. At the lower temperature used in this study (10 °C), the hyperthermic effect of MDMA was not observed and in many cases converted to a hypothermic response. Furthermore, interference with the hyperthermia did attenuate the reduction in 5-HT concentration measured 1 week later. This suggests that antagonism of the hyperthermia produced by MDMA may be a viable mechanism for interference with the long-term neurochemical effects of the drug.

Several agents previously shown to antagonize the neurotoxicity of MDMA were tested for their effect on MDMA-induced hyperthermia. The highly selective 5-HT_2 antagonist, MDL 11,939[3], completely prevented the hyperthermia resulting from the administration of the lowest single dose of MDMA capable of producing neurotoxicity in rats (10 mg/kg). At the dose of MDMA routinely used in our neurotoxicity studies (20 mg/kg) the 5-HT_2 antagonist was protective while only partially antagonizing the hyperthermia. At a still higher dose of MDMA, the antagonist was unable to prevent completely the hyperthermia produced by MDMA, yet neurochemical analysis 1 week later confirmed that the neurotoxicity was completely prevented. In contrast to MDL 11,939, a high dose of the dopamine antagonist, haloperidol, had no effect on the hyperthermia but still significantly attenuated the neurochemical response to MDMA. These data demonstrate that the hyperthermia produced by MDMA is due to the activation of 5-HT_2 receptors rather than D_2 receptors and that interference

with this response by 5-HT_2 antagonists can be dissociated from the blockade of MDMA-induced neurotoxicity. It is also clear that the protective effect of haloperidol does not involve any alteration of MDMA-induced hyperthermia.

Inhibitors of the 5-HT uptake carrier have been shown to block both the acute depletion of 5-HT concentrations by MDMA and its long-term neurotoxic effects[14,15]. Antagonism of the acute effect by 5-HT uptake inhibitors is believed to be related to the ability of these agents to block the carrier-mediated efflux of transmitter initiated by MDMA[15,17]. 5-HT uptake inhibitors would therefore be expected to antagonize the 5-HT_2-mediated hyperthermic response to MDMA by reducing 5-HT release. Other studies have demonstrated inhibition of PCA-induced hyperthermia by 5-HT uptake inhibitors in rats[11] and rabbits[12]. Although MDL 27,777 initially blocked the elevation of body temperature produced by 10 mg/kg MDMA, the blockade was short-lived while at the higher dose of MDMA, the uptake inhibitor was without effect. It is interesting to note that the antagonism of PCA-induced hyperthermia by citalopram or clomipramine was also maintained for only 60 min post-drug administration[11]. This suggests that the blockade of the hyperthermia requires almost total inhibition of the uptake carrier. Therefore even a small decrement in the degree of uptake inhibition may allow sufficient release to produce the increase in body temperature. The extent of uptake inhibition provided by MDL 27,777 does decline a small but significant degree after 2 h as demonstrated in experiments using pretreatment with the inhibitor to protect against 5-HT depletion by *p*-chloroamphetamine[5]. In spite of its minimal effect on MDMA-induced hyperthermia, the uptake inhibitor completely blocked the neurotoxic effects of both doses of MDMA. This indicates that the ability of uptake inhibitors to block the serotonergic deficits produced by MDMA does not involve any modification of the hyperthermia also produced by the drug.

The effect of ambient temperature on the neurotoxicity of MDMA remains to be explained. Perhaps the most obvious interpretation of these results would be that a change in the metabolic activity of the animals at the reduced temperature modifies the metabolism or disposition of the drug. However, the acute depletion of 5-HT by MDMA was not affected by blocking the hyperthermia suggesting that the amount of drug reaching the brain is not altered appreciably. To clearly dissociate the hyperthermic response from the neurotoxicity would require blocking the temperature response while still producing the neurochemical changes.

In conclusion, 5-HT_2 receptors are involved in both the neurotoxic effects of MDMA and the hyperthermia produced by the drug. A possible link between the two effects was suggested at lower doses of MDMA where the 5-HT_2 receptor antagonist MDL 11,939 attenuated both the serotonergic deficits and the hyperthermia. Although the blockade of MDMA-induced hyperthermia and neurotoxicity by reduced ambient temperatures indicates this is a viable explanation for the protective effects of 5-HT_2 antagonists, interference with hyperthermia and neurotoxicity by MDL 11,939 could be dissociated at higher doses of MDMA. The results indicate that the long-term neurochemical and hyperthermic effects of MDMA are independently mediated by 5-HT_2 receptors. The protective effects of the 5-HT uptake inhibitor MDL 27,777 and the dopamine receptor antagonist, haloperidol, were also clearly unrelated to any effect on MDMA-induced hyperthermia. Alternative explanations for the protective effects of these various agents must therefore be sought. In addition, the results indicate that MDMA-induced hyperthermia may be a useful marker of 5-HT_2 receptor activation in studies of the early neurochemical effects of MDMA and its analogs.

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