The relationship between hyperthermia and glycogenolysis in 3,4-methylenedioxymethamphetamine-induced serotonin depletion in rats

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Received 14 January 2004; received in revised form 23 March 2004; accepted 25 March 2004
Available online 5 May 2004

Abstract

Although the exact mechanisms involved in the serotonergic neurotoxicity produced by substituted amphetamines are not completely known, evidence suggests that oxidative and/or bioenergetic stress may contribute in the mechanism of neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA). It has been postulated that MDMA-induced hyperthermia also contributes to the MDMA-induced neurotoxicity. The relationship of MDMA-induced hyperthermia and glycogenolysis in the serotonergic neurotoxicity of MDMA was investigated in the present study. The administration of MDMA (20 mg/kg sc) at an ambient temperature of 24 °C produced hyperthermia and brain glycogenolysis in Postnatal Day (PND)21 and PND70 rats; however, long-term reductions in serotonin (5-HT) concentrations in the striatum were detected only in the PND70 rats. Treatment of PND21 and PND70 rats with MDMA at 17 °C resulted in neither hyperthermia nor glycogenolysis; nevertheless, long-term reductions in 5-HT concentrations were still evident in the PND70 rats treated with MDMA. These results support the conclusion that hyperthermia, as well as glycogenolysis, are neither necessary nor sufficient in the serotonergic neurotoxicity of MDMA.

Keywords: Glycogenolysis; Hyperthermia; Neurotoxicity; MDMA

1. Introduction

3,4-methylenedioxymethamphetamine (MDMA), a ring-substituted amphetamine analog, is widely abused as a recreational drug, and there is concern that the drug may damage serotonergic nerve terminals [17]. MDMA-induced neurotoxicity of serotonergic nerve terminals in rodents and nonhuman primates is evidenced by several biochemical and immunocytochemical findings such as depletion of tissue concentration of serotonin (5-HT) and its major metabolite, 5-hydroxyindoleacetic acid [43,51], decrease in the activity of the enzyme tryptophan hydroxylase [44], reduction in the [3H] paroxetine-labeled 5-HT reuptake sites [2], and reduced immunostaining of 5-HT terminals [38].

Although the exact mechanisms involved in the serotonergic neurotoxicity produced by amphetamine analogs are not completely known, evidence is supportive of a role of oxidative and/or bioenergetic stress in the process. For example, MDMA increases hydroxyl radical formation [11,46,47] and reduces the concentration of the endogenous antioxidants vitamin E and ascorbic acid [48]. The administration of antioxidants also has been shown to attenuate MDMA-induced 5-HT depletion [10,18,48]. The involvement of bioenergetic stress is predicated on the findings that methamphetamine reduces striatal concentration of ATP [9] and administration of energy substrates, i.e., ubiquinone and nicotinamide, attenuates methamphetamine-induced neurotoxicity [49]. In addition, MDMA produces a rapid and transient inhibition of mitochondrial function [8], and the mitochondrial toxin malonate greatly exacerbates MDMA neurotoxicity [35].

The findings that substituted amphetamines decrease brain glycogen [13,19,21] and increase the extracellular concentration of glucose in the brain [13] also are consistent with the view that amphetamines disrupt cellular energetics. MDMA has also been shown to activate glycogen phosphorylase, an enzyme responsible for the breakdown of glycogen, in astroglial-rich primary cultures [40].

Hyperthermia has been postulated to contribute to substituted amphetamine-induced neurotoxicity [7,25,26], and
alterations in ambient temperature at which methamphetamine or MDMA are administered to rats affect the magnitude of neurotoxicity [3–5,16,27,45]. Hyperthermia also may contribute to MDMA-induced glycogenolysis [13].

The purpose of the present study was to examine the relationship between MDMA-induced hyperthermia and glycogenolysis as it relates to the process of MDMA-induced serotonergic neurotoxicity.

2. Methods

2.1. Animal procedures

Male rats of Postnatal Day (PND)70 (300–325 g) and PND21 rats of the Sprague-Dawley strain (Charles River, Portage, MI) were used in the studies. The adult male (PND70) rats were housed three per cage in a temperature- and humidity-controlled room with a 12:12-h light–dark cycle and allowed food and water ad libitum. Pregnant rats (Charles River, Portage, MI) were singly housed, and upon parturition, the offspring in each litter were counted, sexed, and culled to eight pups (four male and four female). At PND21, litters were weaned, and the offspring were grouped four per sex and four per cage. At PND70, litters were weaned, female pups were culled, and male pups were housed three per cage. On the day of the experiment, PND70 and PND21 rats were placed in an environment maintained at 24 or 17 °C. On the day of the experiment, PND70 and PND21 rats were killed by decapitation 7 days following treatment with MDMA, and the striatum was dissected out from the rest of the brain and stored at −80 °C. Tissue samples were homogenized in ice-cold 0.2 N perchloric acid, and the homogenates were then centrifuged at 14,000 rpm for 30 min. Aliquots of the supernatant (20 μl) were injected onto a C-18 reverse phase column connected to a LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN) equipped with a glassy carbon target electrode. The mobile phase for the separation of 5-HT was consisted of 35 mM citric acid, 54 mM sodium acetate, 50 mg/l disodium ethylenediamine tetraacetate, 50 mg/l octane sulfonic acid sodium salt, 3% methanol, and 3% acetonitrile, pH 4.1, pumped at a flow rate of 0.3 ml/min. Peak heights were recorded with an integrator, and quantities of 5-HT were calculated on the basis of known standards.

2.2. Chemicals, drugs, and drug treatment

MDMA was provided by the National Institute of Drug Abuse and was dissolved in 0.15 M NaCl. MDMA was administered at an ambient temperature of 24 °C (unless noted otherwise) to rats housed three per cage (47 × 25 × 20 cm). NADP disodium salt, hexokinase, glucose-6-phosphate dehydrogenase, and amyloglycosidase were purchased from Roche Diagnostics (Chicago, IL). All reagents used in the assay were prepared as described [30].

2.3. Biochemical measurements

2.3.1. Assay of tissue glycogen

The procedure for the preparation of tissue samples and analysis of glycogen content were similar to those described previously [12,13,30]. Briefly, rats were killed by decapitation, and the brains were rapidly removed from the skull and immersed in liquid nitrogen within 12–15 s. The cerebellum was separated from the rest of the brain, and the tissue was stored at −80 °C until analysis. Brain tissue (approximately 250 mg) consisting of the caudal quarter of the left cerebral hemisphere was weighed and homogenized in 3 ml of ice-cold 0.03 N HCl. The homogenate was placed in boiling water for 5 min. Assay tubes contained 300 μl of acetate buffer (pH = 4.6), 100 μl of the homogenate or glycogen standard, and 10 μl of amyloglycosidase or water. The tubes were vortexed and incubated at room temperature for 30 min. After incubation, 1.33 ml Tris buffer (pH = 7.8), 0.66 ml MgCl2 0.6H2O (2 mg/ml), 100 μl ATP (2 mg/ml), and 10 μl NADP (10 mg/ml) were added to each tube. The tubes were vortexed and subjected to centrifugation at 10,000 × g for 5 min. The supernatants were transferred to other tubes, and the fluorescence (excitation 350 nm/ emission 460 nm) was measured in a fluorescence spectrophotometer (Model: F-2000, Hitachi Instruments, Naperville, IL). Hexokinase (10 μg/10 μl) and glucose-6-phosphate dehydrogenase (2 μg/10 μl) were then added, and the tubes were vortexed and incubated at room temperature for 30 min. The fluorescence was again measured. The difference in the fluorescence values was corrected for sample, reagent, and enzyme blank. Glycogen values are reported as glucose equivalents (μmol/g tissue).

2.3.2. Assay of tissue 5-HT

Rats were killed by decapitation 7 days following treatment with MDMA, and the striatum was dissected out from the rest of the brain and stored at −80 °C. Tissue samples were homogenized in ice-cold 0.2 N perchloric acid, and the homogenates were then centrifuged at 14,000 rpm for 30 min. Aliquots of the supernatant (20 μl) were injected onto a C-18 reverse phase column connected to a LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN) equipped with a glassy carbon target electrode. The mobile phase for the separation of 5-HT was consisted of 35 mM citric acid, 54 mM sodium acetate, 50 mg/l disodium ethylenediamine tetraacetate, 50 mg/l octane sulfonic acid sodium salt, 3% methanol, and 3% acetonitrile, pH 4.1, pumped at a flow rate of 0.3 ml/min. Peak heights were recorded with an integrator, and quantities of 5-HT were calculated on the basis of known standards.

2.4. Body temperature measurements

On the day of the experiment, the rats were allowed to acclimate in their cages at 24 or 17 °C for 2 h before body temperatures were measured. Measurements of rectal temperature were made using a telethermometer and a thermister probe. The probe was lubricated with a small amount of petroleum jelly and inserted 5 cm into the rectum of each rat, where it remained for at least 30 s, until a stable temperature was obtained. Measurements were taken every 30 min for a 1.5-h period prior to administration of MDMA (20 mg/kg sc) and for a 1.5-h period at 30-min intervals following the injection of the drug. The change in body temperature was determined by subtracting the body temperature at time 0 from the maximal body temperature recorded after MDMA administration. The rats were killed 7 days later, and the striatum was excised for determination of 5-HT.
2.5. Statistical analysis

The effects of MDMA on brain glycogen and striatal 5-HT were analyzed with a two-way analysis of variance (ANOVA). Multiple pair-wise comparisons were performed using the Student–Newman–Keuls test. The effect of MDMA on body temperature was analyzed using t test. Treatment differences for all the data were considered statistically significant at \( P < 0.05 \).

3. Results

A single injection of MDMA (20 mg/kg sc) produced a significant \(( t = 7.1, P < 0.001)\) increase in body temperature of 1.3 °C in PND21 rats maintained at 24 °C (Table 1). MDMA-induced hyperthermia in PND21 rats at 24 °C was accompanied by a significant \(( P < 0.001)\) 34% decrease in glycogen content in the brain (Fig. 1A).

Maintenance of PND21 rats at a cool ambient temperature of 17 °C not only prevented MDMA-induced hyperthermia but also completely abolished the MDMA-induced glycogenolysis. Indeed, at 17 °C, MDMA treatment produced a significant \(( t = 5.2, P < 0.001)\) decrease in body temperature of PND21 rats (Table 1), and brain glycogen concentrations were not significantly different \(( P = 0.83)\) between vehicle- and MDMA-treated animals (Fig. 1A).

Although hyperthermia and glycogenolysis were elicited in PND21 rats by MDMA at 24 °C, there was no main effect of MDMA on brain 5-HT in PND21 rats maintained at 24 or 17 °C \([ F(1,17) = 0.32, P = 0.58] \) (Fig. 1B).

Treatment of PND70 rats with MDMA (20 mg/kg sc) at 24 °C resulted in a hyperthermic response of 1.5 °C \(( t = 10.4, P < 0.001; \) Table 1) and a significant \(( P < 0.001)\) 40% reduction of brain glycogen (Fig. 2A). However, MDMA treatment failed to alter body temperature \(( t = 2.2, P = 0.053)\) or brain glycogen \(( P = 0.44)\) in PND70 rats maintained at 17 °C (Table 1, Fig. 2A).

Although MDMA-induced hyperthermia and glycogenolysis were abolished in rats maintained at 17 °C, the

| Table 1 |
| Age/ambient temperature (°C) | \( \Delta \) Body temperature (°C) | VEH | MDMA |
| PND 21 | 24 | 0.2 ± 0.0 | 1.3 ± 0.1 * |
| | 17 | 0.1 ± 0.1 | −0.4 ± 0.1 * |
| PND 70 | 24 | 0.2 ± 0.0 | 1.5 ± 0.1 * |
| | 17 | 0.3 ± 0.1 | −0.2 ± 0.2 |

PND21 and PND70 rats were maintained at ambient temperatures of 24 or 17 °C for 2 h prior to and following the administration of MDMA (20 mg/kg sc). Body temperatures were recorded at 30-min intervals, for a 1.5-h period prior to drug treatment and for a 1.5-h period at 30-min intervals following the injection of the drug. The values shown are the difference between the peak body temperature recorded after administration of MDMA and that recorded at the time of administration of MDMA. The values represent the mean ± S.E. of five to six rats. * \( P < 0.05 \) compared with vehicle (VEH)-treated rats.

Fig. 1. Effect of ambient temperature on MDMA-induced glycogenolysis and long-term 5-HT depletion in PND21 rats. (A) Rats were maintained at ambient temperatures of 24 and 17 °C for 2 h prior to administration of MDMA (20 mg/kg sc), and the rats were killed 1 h after drug treatment. The values represent the mean ± S.E. of five to six rats and are expressed in terms of micromoles of glucose liberated from glycogen. (B) Rats were maintained at ambient temperatures of 24 and 17 °C for 2 h prior to administration of MDMA (20 mg/kg sc), and the rats were killed 1 week after drug treatment. The values represent the mean ± S.E. of five to six rats. * \( P < 0.05 \) compared with vehicle (VEH)-treated rats.
magnitude of the MDMA-induced depletion of 5-HT in the striatum of PND70 rats maintained at 17 °C did not differ from that in rats maintained at 24 °C (P = 0.66; Fig. 2B). In PND70 rats maintained at 17 or 24 °C, MDMA treatment resulted in similar reductions of 5-HT concentrations in the striatum of 49% and 45%, respectively.

4. Discussion

Amphetamine analogs have been shown to produce a breakdown of brain glycogen in rodents. The systemic administration of amphetamine has been shown to deplete brain glycogen in mice [21] and rats [32,37]. Parachloroamphetamine, MDMA, and methamphetamine also have been shown to produce glycogenolysis in the rat brain [13,19]. In the present study, MDMA administration resulted in the depletion of brain glycogen in both PND21 and PND70 rats maintained at 24 °C, and these results are consistent with the aforementioned results.

Glycogen represents the primary energy source in the brain [54], and the present results are consistent with the view that MDMA produces a decrease in the energy stores in the brain. Results from previous studies also suggest that amphetamine analogs disrupt cellular energetics in the brain. Administration of MDMA, as well as methamphetamine, causes a rapid and transient inhibition of mitochondrial function [8]. In addition, amphetamine analogs have been shown to affect ATP content and the concentrations of energy substrates and extracellular glucose in the brain [9,13,20,22,31,53]. Finally, pretreatment with nicotinamide has been shown to attenuate the n-amphetamine-induced depletion of striatal ATP in rats [57].

Previous results are suggestive that MDMA-induced glycogenolysis is mediated by 5-HT2 receptors and appears to be associated with the hyperthermic response to the drug. It has been reported that 5-HT stimulates glycogenolysis in slices of cerebral cortex [42]. Moreover, antagonists that suppressed 5-HT-induced glycogenolysis in vitro [42] ultimately were found to be agents with high affinity for the 5-HT2 receptor [39]. It has been shown that 5-HT, 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB), a 5-HT2 agonist, and MDMA, a weak 5-HT2 agonist and a 5-HT-releasing agent, activate glycogen phosphorylase in astroglial-rich primary cultures [40]. Activation of 5-HT2 receptors in vivo also has been shown to produce glycogenolysis in the rat brain [12]. It has been suggested that glycogenolysis induced by amphetamine analogs is due to the energy demands resulting from drug-induced hyperthermia [19].

MDMA-induced hyperthermia has been shown to be dependent on developmental age and ambient temperature [25,26,34], and activation of 5-HT2 receptors is thought to mediate the increase in body temperature produced by MDMA [33,45]. Attenuation of MDMA-induced hyperthermia by administration of a 5-HT2 antagonist or by maintenance of rats at a cool ambient temperature has been shown to be accompanied by attenuation of MDMA-induced glycogenolysis [13]. The present finding that MDMA-induced glycogenolysis was attenuated in PND21 and PND70 rats in which hyperthermia was attenuated at 17 °C, albeit correlative in nature, further supports an association between hyperthermia and glycogenolysis produced by MDMA.

MDMA-induced hyperthermia has been shown to be dependent on developmental age and ambient temperature
neurons [6,7]. MDMA fails to produce an increase in body temperature in PND10 rats but does so in PND21, PND40, and PND70 rats [1,7]. In the present study, MDMA produced an increase in body temperature in PND21 and PND70 rats maintained at 24 °C, and this agrees with the aforementioned studies on the ontogeny of MDMA-induced hyperthermia. MDMA-induced hyperthermia in both PND21 and PND70 rats was absent in rats maintained at 17 °C. These findings are consistent with other reports [7,13,25], in which it was demonstrated that MDMA failed to elicit a hyperthermic response in rats maintained at 10, 17, and 20 °C.

Although hyperthermia per se is not toxic to 5-HT neurons [7], it has been shown to contribute to the neurotoxicity of amphetamine derivatives, including MDMA [14,15,27,45]. Drugs that have been shown to attenuate MDMA-induced hyperthermia have been shown to afford protection against MDMA-induced 5-HT neurotoxicity [14,15,26].

However, MDMA-induced 5-HT neurotoxicity can be dissociated from MDMA-induced hyperthermia. In the present study, PND70 rats injected with MDMA at 17 °C exhibited 5-HT neurotoxicity in the absence of a hyperthermic response. Moreover, MDMA produces long-term 5-HT depletion in rats maintained at 4 °C—a temperature at which MDMA induces hyperthermia [7]. Additionally, the hyperthermic response to MDMA can be dissociated from MDMA-induced 5-HT neurotoxicity. For example, fluoxetine and ascorbic acid do not alter the hyperthermic response to MDMA, yet these agents afford protection against the depletion of brain 5-HT [26,48]. In addition, MDMA induced a hyperthermic response in PND21 rats in the present study, which was similar to that in PND70 animals, yet there was no long-term depletion of 5-HT in the PND21 rats. This finding is in agreement with a previous report [1].

Developing rodents have been shown to be resistant to the neurotoxic effects of amphetamine derivatives. Parchloroamphetamine and methamphetamine do not produce long-term reductions in dopamine or 5-HT when administered to neonatal rats [23,41]. Moreover, MDMA administration does not result in a reduction in 5-HT content or 5-HT reuptake sites in neonatal rats [6,7]. In addition, the repeated administration of MDMA to pregnant rats does not produce deficits in the development of the serotonergic system of the offspring [1,50]. MDMA administration does not produce toxicity in neonatal rats up to PND28; and the time of onset of susceptibility to MDMA-induced 5-HT depletion has been placed between PND 28 and PND35 [1,6,7]. Adult levels of neuronal dopamine are not reached until PND30 [36], and it has been suggested that the lack of sensitivity to MDMA in neonatal rats is due to a failure of MDMA to elevate dopamine sufficiently to produce oxidative stress [1]. Indeed, consistent with this view is the finding that the administration of L-DOPA to PND21 rats imparts vulnerability to MDMA-induced neurotoxicity [1].

The lack of maturation of CNS dopamine systems is only one possible explanation for the resistance of PND 21 rats to MDMA-induced 5-HT neurotoxicity. It has been suggested that a metabolite of MDMA, rather than MDMA itself, is responsible for the long-term depletion of 5-HT [28,29]. In view of the ontogenic pattern of cytochrome P450 enzymes in the postnatal rat [58], the metabolism of MDMA in PND 21 rats may not be sufficient to generate a proposed reactive toxicant.

The lack of 5-HT toxicity in PND 21 rats also may be due to differences in cellular respiration and energy metabolism in immature and adult rats. Cellular respiration and energy metabolism in the neonatal rat is primarily anaerobic, whereas in the adult rat, it is aerobic [52]. This finding, together with the relative abundance of glycogen and lack of neuronal mitochondria in neonatal rats, has been suggested to account for the resistance of immature rats to ischemia-related damage [24,55,56]; such factors also may contribute to the resistance of PND 21 rats to MDMA-induced toxicity.

Although MDMA-induced long-term 5-HT depletion was not observed in PND21 rats, MDMA did elicit hyperthermia and glycogenolysis in these animals. It is evident that neither hyperthermia nor glycogenolysis is sufficient to promote MDMA-induced 5-HT neurotoxicity. It is also evident that MDMA-induced hyperthermia and glycogenolysis are not necessary for the expression of MDMA-induced depletion of 5-HT. This conclusion is based on the finding that administration of MDMA to PND70 rats maintained at 17 °C failed to alter body temperature or glycogen concentrations, but striatal concentrations of 5-HT in these animals were reduced by MDMA treatment.

The results of the present study document further an association between MDMA-induced hyperthermia and glycogenolysis in the rat brain. Although both hyperthermia and glycogenolysis may exacerbate MDMA-induced 5-HT neurotoxicity, both appear to be neither necessary nor sufficient for the long-term depletion of brain 5-HT produced by MDMA.

Acknowledgements

This study was supported by the grant USPHS DA07427.

References


