Acute Toxicity of 3,4-Methylenedioxymethamphetamine (MDMA) in Sprague–Dawley and Dark Agouti Rats

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Received 24 July 1998; Revised 9 November 1998; Accepted 15 February 1999

MALPASS, A., J. M. WHITE, R. J. IRVINE, A. A. SOMOGYI AND F. BOCHNER. Acute toxicity of 3,4-methylenedioxymethamphetamine (MDMA) in Sprague–Dawley and Dark Agouti rats. PHARMACOL BIOCHEM BEHAV 64(1) 29–34, 1999.—Ingestion of MDMA (“ecstasy”) by humans can cause acute toxicity manifested by hyperthermia and death. Demethylenation of MDMA is catalyzed by cytochrome P-450 2D6 (CYP2D6) and cytochrome P-450 2D1 (CYP2D1) in humans and rats, respectively, and is polymorphically expressed. It has been proposed that CYP2D6 deficiency may account for the unexplained toxicity of MDMA. The female Dark Agouti rat is deficient in CYP2D1, and serves as a model for the human poor metabolizer. We investigated thermogenic and locomotor actions of MDMA in adult female Sprague–Dawley (CYP2D1 replete) and Dark Agouti rats. MDMA (2, 5, and 10 mg/kg) and saline were injected subcutaneously at ambient temperatures of 22 and 31°C. There was no difference in core temperature responses between the two rat strains. Hypothermia occurred in the first 30 min and temperature elevation thereafter. MDMA increased locomotor activity in Sprague–Dawley but not in Dark Agouti rats. However, MDMA had pronounced lethal effects at 31°C ambient in the Dark Agouti rats only. We conclude that the poor metaboliser phenotype may predispose to lethality, but the mechanism is as yet unknown. © 1999 Elsevier Science Inc.

3,4-methylenedioxymethamphetamine (MDMA)  Body temperature  Acute toxicity  Locomotor activity
CYP2D1 polymorphism  Sprague–Dawley/Dark Agouti rat

3, 4- METHYLENEDIOXYMETHAMPHETAMINE (MDMA, “Ecstasy”) is an illicit drug with significant abuse potential. Ingestion of MDMA in humans has been associated with severe adverse reactions and unexplained deaths in a small proportion of users following a single dose (3,10). This acute toxicity is characterized by hyperthermia, and subsequent rhabdomyolysis and disseminated intravascular coagulation (13,28). In rats, MDMA also causes temperature elevation, which is dose dependent, and which is exaggerated at high ambient temperatures (7,8,13). This is important, as MDMA is often consumed at night clubs and dance parties, which are characterized by hot, crowded conditions. MDMA causes increased serotonin release in vitro (17,29), and the hyperthermic effects may, therefore, be a consequence of acutely elevated brain serotonin concentrations, as this transmitter has been shown to be involved in temperature regulation (27). In addition, MDMA also causes increased locomotor activity (7,8,11,13,23,24,34). The mechanisms of these effects are unclear, and may involve both serotonergic (2) and/or dopaminergic systems (11,19,33).

One of the elimination pathways of MDMA is demethylation to dihydroxymethamphetamine (DHMA) (14). This reaction is mediated via the cytochrome P450 2D6 (CYP2D6) enzyme in humans (14), and is expressed polymorphically. Approximately 10% of Caucasians lack the functional activity of this enzyme, and are classified as poor metabolisers of substrates of this CYP isofrom (12). The remainder of the population is classified as extensive metabolizers. It has been proposed that a deficiency in this enzyme may result in substantially impaired elimination of MDMA, leading to higher and sustained concentrations of the parent drug in the body and increased toxicity, and hence, could possibly explain the seemingly random deaths that occur in humans (5,12,30).

The female Sprague–Dawley and Dark Agouti rats are the animal counterparts of the human extensive and poor metab-
oliser phenotype, respectively (20,22), with the corresponding enzyme being CYP2D1 (1,4,35). In an extensive study, Chu and co-workers (4) showed that the female Dark Agouti accumulated MDMA in brain and blood to levels several fold higher than Sprague–Dawleys treated with identical doses of MDMA. Thus, it is clear that the enzyme deficiency in the Dark Agouti rat results in higher tissue concentrations of MDMA, and that this may be reflected in greater toxicity. Colado and co-workers (5) reported that the hyperthermic response to MDMA was indeed enhanced in female Dark Agouti rats but not in the male Dark Agouti, and suggested that the human poor metabolizer phenotype was more susceptible to the acute toxicity of MDMA. The male Dark Agouti rat, however, is an inappropriate control, because its capacity to metabolize CYP2D1 substrates is also partly deficient and is less than that of the Sprague–Dawley (31). Thus, the effect of the genetic polymorphism of CYP2D6/2D1 expression and of the deficient demethylation (which occurs in the female Dark Agouti but not Sprague–Dawley rats) on the acute effects of MDMA in vivo has not been satisfactorily examined.

The present study compared the effects of MDMA on thermoregulation and locomotor activity in female Dark Agouti and female Sprague–Dawley rats to evaluate the role of the CYP2D1 enzyme in mediating MDMA toxicity. Males were not studied because of the documented gender differences in demethylation (to form MDA) (4) and temperature responses to MDMA (25). The hypothesis to be tested was that the Dark Agouti strain, which is deficient in CYP2D1, would be more susceptible to hyperthermia and the enhanced locomotor effect of MDMA than the Sprague–Dawley rat, and that these responses would be exaggerated at elevated ambient temperatures.

**METHOD**

Adult female Sprague–Dawley (weight 230–370 g) and aged matched Dark Agouti (weight 180–240 g) rats were used. They were housed four to a cage in a controlled environment (20–24°C, 12 L: 12 D cycle) during acclimatization, but were singly housed during the experiments to avoid the hyperthermic effect of group housing (6). Standard rat chow and water were available ad lib. All animals were allowed a minimum of 2 weeks to acclimatize to the laboratory. During this time they were handled daily and trained to accept the temperature probe. Experiments were not commenced until they were handled daily and trained to accept the temperature probe. Throughout this period the ambient temperature, and at 0, 30, 60, 90, 120, 150, 180, 240, 300, and 360 min postinjection. Throughout this period the ambient temperature was maintained between 29 and 33°C for the duration of the experimental session. Sprague–Dawley (n = 8) and Dark Agouti rats (n = 8) received 2, 5, and 10 mg/kg of MDMA, plus a saline control injection, in random dose order, separated by at least 48 h. All injections were given between 0930 and 1230 h. Rectal temperature was determined 30 min prior to drug administration, and at 0, 30, 60, 90, 120, 150, 180, 240, 300, and 360 min postinjection. Throughout this period the ambient temperature was maintained at 22°C, within a range of 20–24°C.

**Effect of MDMA on temperature at 22°C.** Animals were placed in an environmental chamber, the temperature of which was maintained between 29 and 33°C for the duration of the experimental session. Sprague–Dawley (n = 8) and Dark Agouti rats (n = 8) were each administered three doses of MDMA (2, 5, and 10 mg/kg), plus a saline control injection, in random dose order, separated by at least 48 h. All injections were given between 0930 and 1230 h. Rectal temperature was determined 30 min prior to drug administration, and at 0, 30, 60, 90, 120, 150, 180, 240, 300, and 360 min postinjection. Throughout this period the ambient temperature was maintained at 22°C, within a range of 20–24°C.

**Effect of MDMA on locomotor activity at 22 and 31°C.** To assess the influence of MDMA on locomotor activity, the animals undergoing core temperature readings were placed in the locomotor activity chambers for a 30-min period, beginning 30 min after MDMA administration. These chambers were maintained at the designated temperature during this period, and the total distance travelled was recorded.

**Data and Statistical Analysis**

For body temperature, the following variables were calculated: (a) the peak change (increase or decrease), and (b) the area under the body temperature–time curve (AUC) from 0 to 360 min, calculated by the linear trapezoidal method. The AUC provides a measure of the integrated temperature change over the entire experimental period. Changes in body temperature were frequently biphasic, with an initial decrease followed by an increase. For this reason, maximum hypothermic and hyperthermic changes were analysed separately. For locomotor activity the total distance travelled in the 30-min period was calculated. Dose–response was assessed by analysis of variance (ANOVA), followed by post hoc analysis by Tukey’s multiple comparison test. The minimum level for statistical significance was p < 0.05. Results are expressed as mean ± standard error of the mean (SEM). The influence of the order of drug treatment on dose effects was tested by two-way ANOVA, and no significant order effects were observed. p-Values ranged from 0.056 to 0.840.

**RESULTS**

**Body Temperature Responses at 22°C**

Sprague–Dawley. A dose-dependent initial hypothermic response, which occurred in the first 30 min, was observed (Fig. 1A), with the effect of the highest dose (10 mg/kg) of MDMA being significantly different (p < 0.05) from saline. The subsequent increase in temperature was not dose dependent (Fig. 1B). There was no difference (p > 0.05) in the area
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under the temperature–time curve between any of the doses, including the control (Fig. 2A).

**Dark Agouti.** There was no dose-dependent initial hypothermic effect (Fig. 1A). Following administration of 10 mg/kg MDMA, a biphasic response was noted. Hypothermia was observed in the first 30 min postinjection, after which the body temperature increased. There was a greater hyperthermic response at 10 mg/kg compared to 2 and 5 mg/kg ($p < 0.01$, Fig. 1B). There was no difference ($p > 0.05$) in the area under the temperature–time curve between any of the doses, including the control (Fig. 2B).

There were no statistically significant differences ($p < 0.05$) in the magnitude of the hypo- or hyperthermic responses or area under the temperature–time curve between the strains at any of the doses tested (Fig. 2).

**Body Temperature Responses at 31°C**

**Sprague–Dawley.** No significant decrease in initial temperature was observed for any of the doses (Fig. 1A), and the hyperthermic effect was not significantly different between any of the doses and the saline control (Fig. 1B). However, the area under the temperature–time curve significantly increased with dose (Fig. 2A). There were no deaths in this strain.

**Dark Agouti.** There was an inverse relationship between MDMA dose and hypothermia in which the control and 2-mg/kg doses were significantly ($p < 0.05$) different to the 5- and 10-mg/kg doses (Fig. 1A). There was a dose-dependent increase in body temperature, with the largest change being approximately 2.5°C at 10 mg/kg MDMA (Fig. 1B). At this dose the exact magnitude of temperature elevation was difficult to determine because the first two animals died 1–2 h after MDMA administration, and this dose was, therefore, not further investigated. The area under the temperature–time curve was significantly greater ($p < 0.05$) at 5 mg/kg compared to the control and 2 mg/kg.

When compared to Sprague–Dawley rats, there was a greater decrease in temperature in the Dark Agouti rats at 0 and 2 mg/kg MDMA (Fig. 1A). In addition, the area under the temperature–time curve was significantly greater for the Sprague–Dawley strain compared to the Dark Agouti animals for the saline control, and 2 mg/kg doses (Fig. 2).

**Locomotor Activity**

At 22 and 31°C there was a dose-dependent increase in locomotor activity in the Sprague–Dawley but not in the Dark Agouti strain (Fig. 3). The Sprague–Dawley rats showed a significantly greater increase in locomotor activity at 5 and 10 mg/kg compared to the Dark Agouti rats at 22°C. At 31°C, there was no difference in locomotor activity between the two strains given 2 or 5 mg/kg. The two rats that died exhibited substantially reduced activity consistent with their premorbid state.

**DISCUSSION**

We have confirmed the work of others that the acute effects of MDMA in the rat are manifested, in part, by disruption in thermoregulation (5,7,8,13,25,26) and increased locomotor activity (7,8,16,34), and that the thermoregulatory
changes are exaggerated at higher ambient temperatures (8,13). All of the previously reported work has been carried out in rat strains other than the female Dark Agouti, except for the work of Colado et al. (5), who compared the effects and disposition of MDMA in male and female Dark Agouti animals. We are not aware that an appropriate strain comparison (Sprague–Dawley against Dark Agouti) to examine the effect of the CYP2D1 polymorphism has been hitherto reported.

One of the major differences in outcomes between the two strains was the lethality in the Dark Agouti rats. The highest dose of MDMA could not be assessed in this strain at the 31°C ambient temperature. In contrast, there was no lethality among the Sprague–Dawley rats. This would appear to confirm the hypothesis that CYP 2D1/2D6 deficiency predisposes to MDMA toxicity (5,30,32). The fact that the increased lethality occurred only at the high ambient temperature suggests that it may be a result of hyperthermia.

The effect of MDMA on thermoregulation was complex and the response pattern appears to be qualitatively different in the two strains. The Sprague–Dawley group showed a dose-dependent decrease in temperature at 22°C, but not at 31°C, whereas in the Dark Agouti rats, hyperthermia occurred at 22°C only with 10 mg/kg, and there was an inverse relationship between MDMA dose and hypothermia at 31°C. At 31°C, there was a nonsignificant increase in temperature in the Sprague–Dawley rats, but a dose-related increase in the Dark Agouti strain. The interstrain comparisons showed that there was no difference in hypo- or hyperthermia or the AUC between the two groups at 22°C. At 31°C, the Dark Agouti strain showed a greater hypothermic effect than the Sprague–Dawley animals, which in turn, had a larger AUC at 0 and 2 mg/kg than other group. Thus, overall, it appears that there is little difference in temperature responses to MDMA between the two strains at the doses given in this study, and our data do
not support a role for differential rates of CYP2D1-mediated metabolism of MDMA influencing the thermogenic effects.

The biphasic response in temperature at 22°C has also been noted by McNamara et al. (25) and Gordon (13), but not by other authors (5,7–9,25), who may have missed the decline in temperature because most workers have not measured temperature in the first 30 min after MDMA administration. The mechanism of this response is not clear. A large dose (10 µg) of serotonin injected into the third ventricle or anterior hypothalamus of the conscious monkey caused hypothermia (27), which may occur because of transient overload of serotonin receptors (26). A lower dose of 5 µg caused hyperthermia (27). Ambient temperature has a dramatic influence on the direction and magnitude of MDMA-induced core body temperature changes. Hypothermia has been reported at low (about 10°C) ambient temperatures (7,13) in the Sprague-Dawley strain and marked hyperpyrexia at 30°C, with some of the animals dying (13).

Thus, there was little difference between the strains in the thermal response to MDMA. The difference in lethality could be explained by a relatively steeper dose–response curve for hyperthermia among Dark Agouti rats. It is also possible that lethality in Dark Agouti rats was due to some (unknown) mechanism in addition to the effects on body temperature.

The existence of multiple differences in the actions of MDMA between the strains is supported by the locomotor activity data. The Sprague-Dawley group showed the expected dose-dependent increase in activity at both ambient temperatures, but there was little response in the Dark Agouti rats. There has been a consistent increase in locomotor activity in response to MDMA (11,34), and where examined, this has been dose dependent (7,9,19,23,24). All of the previous studies have been carried out in rat strains other than the female Dark Agouti and at ambient temperatures of about 22°C, except for Dafters (7), who additionally examined the effect of MDMA at 11°C. We are not aware that strain differences in locomotor activity at different ambient temperatures have been previously reported. Our results lend weight to the conclusions of Dafters (7) that there is a dissociation between the thermic and kinetic effects of MDMA.

The pharmacokinetics and metabolism of MDMA are complex, and make the results of in vivo studies very difficult to interpret. In the rat, MDMA undergoes male-specific cytochrome P450-mediated N-demethylation (4) to form 3,4-methylenedioxyamphetamine (MDA), whose pharmacological activity is similar to MDMA (5,34), and two CYP2D1-mediated demethylation reactions to yield 3,4-dihydroxymethamphetamine (DHMA) (22) and 2,4,5-trihydroxymethamphetamine (THMA) (21), which is toxic to both serotonin and dopamine neurones (18). THMA is formed from the precursor ring hydroxylated metabolite 6-hydroxy-3,4-methylenedioxyamphetamine (6-OHMDMA). In vivo, the relative contribution of the demethylation pathway to the overall clearance and acute toxicity of MDMA is not known. In a comprehensive study to compare the disposition and brain serotonin-depleting effect of MDMA in male and female Sprague-Dawley and female Dark Agouti rats, Chu and co-workers (4) found that at 5 and 10 mg/kg MDMA, despite brain and plasma concentrations of MDMA and MDA being significantly higher in the female Dark Agouti group, serotonin depletion was attenuated in the female Dark Agouti rat. Brain concentrations of 6-OHMDMA were significantly greater in the female Dark Agouti rats. These workers concluded that the acute serotonin depletion caused by MDMA could also be due to a CYP2D-catalyzed metabolite.

The major thrust of this study was to determine whether the deficiency in CYP2D6 enzyme activity might enhance acute MDMA toxicity and this then could explain the random deaths that occur in humans. The results here suggest that if this is so, it is not reflected in a simple increased hyperthermic response to the drug. A better understanding of these mechanisms may come about through the study of the metabolites of MDMA and comparison of these with the actions of the parent drug.

ACKNOWLEDGEMENTS

This study was supported by a grant-in-aid from the Research Review Committee of The Royal Adelaide Hospital.

REFERENCES


