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ORIGINAL INVESTIGATION

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Role of the serotonin 5-HT_{2A} receptor in the hyperlocomotive and hyperthermic effects of (+)-3,4-methylenedioxymethamphetamine

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Abstract *Rationale:* Contradictory evidence exists regarding the role of the 5-HT_{2A} receptor (5-HT_{2A}R) in hyperactivity and hyperthermia elicited by the substituted amphetamine (+)-3,4-methylenedioxymethamphetamine. *Objectives:* The present studies examined the ability of the selective 5-HT_{2A}R antagonist M100907 to block hyperactivity and hyperthermia produced across the (+)-MDMA dose-effect curve. *Methods:* Male rats were pretreated with M100907 (0, 0.25, 0.5, 1, and 2 mg/kg) followed by treatment with (+)-MDMA (0–12 mg/kg); activity was recorded for 90 min followed by determination of rectal temperature. Additionally, we investigated the ability of M100907 (0 and 0.5 mg/kg) to reverse hyperthermia elicited by (+)-MDMA (12 mg/kg). *Results:* The first study demonstrated that M100907 attenuated hyperactivity in the periphery of the monitor and eliminated rearing induced by (+)-MDMA (3 mg/kg) with no effect on basal activity. In two subsequent studies, (+)-MDMA (0–12 mg/kg) dose-dependently increased peripheral activity and rearing and produced hyperthermia. Pretreatment with M100907 decreased peripheral activity evoked by (+)-MDMA, right-shifted the dose-effect curve for rearing, and blocked (+)-MDMA-induced hyperthermia, while having no effect when administered alone. A final study demonstrated the ability of M100907 (0.5 mg/kg) to reverse hyperthermia produced by (+)-MDMA (12 mg/kg). *Conclusions:* These results suggest that the 5-HT_{2A}R contributes to the generation of peripheral hyperactivity and rearing and, especially, the hyperthermia evoked by (+)-MDMA and that 5-HT_{2A}R antagonists should be further investigated as treatments for the psychological and hyperthermic effects of (±)-MDMA.

Keywords MDMA · Hyperactivity · Hyperthermia · M100907

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Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is a substituted amphetamine that was originally patented by Merck in 1914 as an appetite suppressant but never marketed (Cami et al. 2000). In recent years (±)-MDMA, the form used "on the street," has emerged as a popular recreational drug due to its unique subjective effects, including increased confidence, elevation of mood, and a sense of closeness with other people (Morgan 2000). Upon acute administration, (±)-MDMA has been shown to induce effects such as hyperthermia, nausea, and jaw clenching, while withdrawal from repeated exposure has been associated with depression, memory impairments, and sleep disorders (Dar and McBrien 1996; Davison and Parrott 1997; Morgan 2000). The behavioral and physiological effects of (±)-MDMA in animals include an increase in locomotor activity, particularly in the periphery of the activity chamber (Paulus and Geyer 1992), serotonin (5-HT) syndrome behaviors (Spanos and Yamamoto 1989), and hyperthermia (Dafters 1994). These effects of (±)-MDMA are thought to be mediated through the release of monoamines via reversal of serotonin, dopamine (DA), and norepinephrine transporters as well as vesicular transporters (Rudnick and Wall 1992; Rothman et al. 2001; Mlinar and Corradetti 2003), although (±)-MDMA does appear to have modest affinity for some 5-HT receptors, including 5-HT_1 (5-HT_1R) and 5-HT_2R (Battaglia et al. 1988).

Our laboratory is interested in the role of 5-HT_1R and 5-HT_2R in the behavioral and physiological effects of the more potent isomer (+)-MDMA. While a 5-HT_{1A}R antagonist was ineffective (McCreary et al. 1999), 5-HT_{1B}R

antagonists effectively attenuated hyperactivity induced by a low dose of (+)-MDMA (McCreary et al. 1999) or (±)-MDMA (Fletcher et al. 2002). The 5-HT_2R family also appears to be involved in the production of (±)-MDMA-induced hyperactivity, since the non-selective 5-HT_2R antagonist ritanserin decreased hypermotility produced by a high dose of (±)-MDMA (Kehne et al. 1996b). Studies employing more selective antagonists suggest that the 5-HT_{2A}R and the 5-HT_{2C}R may play different roles in the generation of hyperactivity produced by (+)-MDMA or (±)-MDMA. For example, selective 5-HT_{2C}R antagonists have been shown to greatly increase hyperactivity induced by a low dose of (+)-MDMA (Herin and Cunningham 2001; Bankson and Cunningham 2002) or (±)-MDMA (Fletcher et al. 2002). These data suggest that indirect activation of the 5-HT_{2C}R serves to self-limit MDMA-evoked hyperactivity and that the 5-HT_{2C}R has a similar role in modulation of hyperactivity evoked by low doses of (+)-MDMA and (±)-MDMA.

The role of the 5-HT_{2A}R in MDMA-evoked hyperactivity is more difficult to discern because a comprehensive analysis of the ability of a selective 5-HT_{2A}R antagonist to alter horizontal hyperactivity and rearing induced by MDMA has not been undertaken. Pretreatment with a single dose of antagonists of varying selectivity for the 5-HT_{2A}R attenuated (Kehne et al. 1996b; Fantegrossi et al. 2003), enhanced (Fantegrossi et al. 2003) or failed to alter (Bankson and Cunningham 2002) horizontal activity induced by (+)-MDMA or (±)-MDMA. Similarly, the literature is inconsistent regarding the role of 5-HT_{2A}R in hyperthermia evoked by (+)-MDMA or (±)-MDMA (Mechan et al. 2002; Fantegrossi et al. 2003), a potentially life-threatening consequence of (±)-MDMA use in humans, which can lead to disseminated intravascular coagulation, rhabdomyolysis, organ failure, and death (Dar and McBrien 1996). These conflicting studies warranted a comprehensive, systematic examination of the role of 5-HT_{2A}R in MDMA-evoked hyperactivity and hyperthermia.

The purpose of the present study was to thoroughly investigate the role of the 5-HT_{2A}R in the behavioral and physiological effects of the more potent isomer (+)-MDMA. In these studies, we utilized the selective 5-HT_{2A}R antagonist M100907, which has 100-fold selectivity for 5-HT_{2A}R versus 5-HT_{2C}R (Kehne et al. 1996a), to determine its ability to block hyperactivity and hyperthermia induced by (+)-MDMA (0–12 mg/kg) as well as reverse hyperthermia produced by the substituted amphetamine. Hyperactivity in the periphery of the chamber was measured since (+)-MDMA and (±)-MDMA primarily increases locomotion in this area of the activity monitor (Paulus and Geyer 1992; McCreary et al. 1999), and rearing was determined since the substituted amphetamine has been shown to enhance rearing (McCreary et al. 1999). As indicated, incomplete and contradictory evidence exists regarding the role of 5-HT_{2A}R in horizontal hyperactivity and hyperthermia and our goal was to establish the efficacy of multiple doses of (+)-MDMA to evoke hyperactivity, rearing and hyperthermia in the presence or absence of multiple doses of the 5-HT_{2A}R antagonist M100907. In addition, we are the first to study the ability of a 5-HT_{2A}R antagonist to reverse (+)-MDMA-evoked hyperthermia. Overall, our data suggest that activation of 5-HT_{2A}R plays an important role in the generation of hyperactivity, rearing and, especially, hyperthermia induced by (+)-MDMA.

Materials and methods

Animals

Male Sprague–Dawley rats (Harlan Sprague–Dawley, Inc., Indianapolis, Ind., USA) weighed 225–275 g at the beginning of the study. The rats were housed either two or four per cage in standard plastic rodent cages in a temperature (21–23°C) and humidity (55–65%) controlled environment under a 12-h light/dark cycle (lights on 0700 hours). Animals were acclimated to the colony for at least 1 week prior to the start of experimental sessions and separate cohorts of animals were used for each study. All experiments were conducted during the light phase of the light/dark cycle (1200–1800 hours) and were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and with approval from the Institutional Animal Care and Use Committee.

Behavioral apparatus

Locomotor activity was quantified using a modified open-field activity system under low-light conditions (San Diego Instruments, San Diego, Calif., USA). Each enclosure consisted of a clear Plexiglas open field (40×40×40 cm) and a 4×4 photobeam matrix located 4 cm above the cage floor for the measurement of horizontal activity; each monitor was housed within sound-attenuating chambers. Activity recorded in the inner 16×16 cm of the open field was counted as central activity, while activity in the outer 12 cm band registered peripheral activity. A second horizontal row of 16 photobeams located 16 cm from the floor allowed the measurement of rearing. Activity counts were made by the control software (Photobeam Activity Software; San Diego Instruments) and stored for statistical evaluation. Video cameras located above the enclosures were used to monitor activity continuously without disruption of behavior.

Temperature apparatus

Animal temperature was measured in experiments 2 and 3 using a thermometer (Model 43TA; Yellow Springs Instrument Co., Yellow Springs, Ohio, or Physitemp Model TH-8; Physitemp Instrument, Inc., Clifton, N.J., USA) connected to a rectal probe that was coated with petroleum jelly and inserted 4 cm into the rectum. Animals were lightly restrained until a stable temperature was obtained (30–60 s maximum). Measurements of rectal temperatures

were taken following the completion of behavioral testing and return to the animal colony (experiment 2) or in the behavior laboratory (experiment 3).

Drugs

(+)-MDMA [(+)-3,4-methylenedioxymethamphetamine] HCl salt (National Institute on Drug Abuse, Research Triangle, N.C., USA) was dissolved in 0.9% NaCl. M100907 [*R*-(+)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol] (synthesized by Thomas Ullrich and Kenner Rice, National Institutes of Health) was dissolved in a 10% solution of 2-hydroxypropyl-β-cyclodextrin (Cyclodextrin Technologies, Gainesville, Fla., USA) in 0.9% NaCl. All doses of (+)-MDMA and M100907 were chosen based on previous research (Kehne et al. 1996b; Hitchcock et al. 1997; Wettstein et al. 1999; Bankson and Cunningham 2002; Fantegrossi et al. 2003; McCreary et al. 2003) and injections were given either IP (M100907) or SC [(+)-MDMA] in a volume of 1 ml/kg. Doses of all drugs refer to the weight of the salt.

Behavioral procedures

Experiment 1: Effects of M100907 (0, 0.5, 1, and 2 mg/kg) pretreatment on hyperactivity induced by a low dose of (+)-MDMA (3 mg/kg)

Rats were randomly assigned to one of eight groups (n=10 rats per group) and habituated to the activity chamber for 3 h/day for the 3 days prior to the experiment. On the test day, rats were placed in the activity monitors for 15 min before an injection of vehicle (1 ml/kg, IP) or M100907 (0.5, 1, and 2 mg/kg, IP), followed 45 min later by an injection of saline (1 ml/kg, SC) or (+)-MDMA (3 mg/kg, SC). Measurements of locomotor activity began immediately after the second injection and were taken for a total of 90 min.

Experiment 2: Effects of M100907 (0, 0.25, 0.5, and 1 mg/kg) pretreatment on the ability of (+)-MDMA (0–12 mg/kg) to induce hyperactivity and hyperthermia

Rats were habituated to the activity chambers for 3 h/day for the 3 days prior to the experiment. This study was conducted in two cohorts of rats that were randomly assigned to groups. On the test day, animals in the first cohort (n=8–16 per group) were placed in activity monitors 15 min before injection with vehicle (1 ml/kg, IP) or M100907 (1 mg/kg, IP), followed 45 min later by an injection with saline (1 ml/kg, SC) or (+)-MDMA (2, 4, 8, and 12 mg/kg, SC); the second cohort (n=7–13 per group) received vehicle or lower doses of M100907 (0.25 and 0.5 mg/kg, IP), followed 45 min later by saline or (+)-MDMA (2, 4, 8, and 12 mg/kg, SC). Measurement of locomotor activity began immediately following the second injection and lasted for a total of 90 min. Immediately following the termination of the test session, rats were removed from monitors, and rectal temperature was measured [90 min following (+)-MDMA administration]. The temperature of the laboratory ranged from 23°C to 25°C during this experiment.

Experiment 3: Ability of M100907 (0 and 0.5 mg/kg) to reverse hyperthermia produced by a high dose of (+)-MDMA (12 mg/kg)

Following random assignment to one of four groups (n=8 per group), the rectal temperature of each animal was measured in the laboratory (23°C ambient temperature), followed immediately by treatment with either saline (1 ml/kg) or (+)-MDMA (12 mg/kg, SC). Animals were injected 60 min later with vehicle or M100907 (0.5 mg/kg, IP). Rectal temperature was measured in each animal every 15 min following the first injection for a total of 4 h.

Statistical analysis

Peripheral activity and rearing counts were summed for each individual rat across the 90-min test session. All data are presented as mean activity counts or mean temperature (±SEM). A two-way ANOVA for independent groups was used to analyze the effects of pretreatment with M100907 (factor 1) and (+)-MDMA treatment (factor 2) on either activity or temperature (experiments 1 and 2). A three-way ANOVA was used to analyze the effects of (+)-MDMA administration (factor 1), M100907 challenge (factor 2), and time (factor 3) on temperature (experiment 3) with a between-subjects design for the first two factors and repeated measures for the third factor (time). Because group comparisons were specifically defined prior to the start of the experiment, a priori planned pairwise comparisons were then made with the Student–Newman–Keuls test (SAS for Windows, Version 8.1) to determine statistical differences between the treatment groups (experimentwise α=0.05). This approach to statistical analysis is supported by a number of statisticians (Keppel 1991; Sheskin 2000).

Results

Effects of M100907 (0, 0.5, 1, and 2 mg/kg) pretreatment on peripheral hyperactivity and rearing induced by a low dose of (+)-MDMA (3 mg/kg)

A main effect of M100907 pretreatment [$F(3,79)=9.34$, $P<0.0001$] and (+)-MDMA treatment [$F(1,79)=167.65$, $P<0.0001$], and a pretreatment×treatment interaction [$F(3,79)=7.74$, $P=0.0001$] were observed for total peripheral activity summed across the 90-min test. As shown in Fig. 1a, (+)-MDMA (3 mg/kg) significantly increased peripheral locomotor activity, and pretreatment with M100907

(0.5, 1, and 2 mg/kg) attenuated (+)-MDMA-evoked hyperactivity ($P<0.05$) without altering basal levels of activity ($P>0.05$). The degree to which each dose of M100907 (0.5, 1, and 2 mg/kg) suppressed (+)-MDMA-evoked hyperactivity was equivalent and levels of activity were not decreased to the control baseline.

A main effect of M100907 pretreatment [$F(3,79)=4.39$, $P=0.0068$] and (+)-MDMA treatment [$F(1,79)=6.66$, $P=0.0119$], and a pretreatment×treatment interaction [$F(3,79)=4.58$, $P=0.0054$] were observed for total rearing summed across the 90-min test. Rearing was significantly increased by (+)-MDMA (3 mg/kg) and all doses of M100907 (0.5, 1, and 2 mg/kg) significantly and completely blocked (+)-MDMA-induced rearing to control levels (Fig. 1b; $P<0.05$). Again, basal rearing was unaffected by any dose of M100907 (0.5, 1, and 2 mg/kg) ($P>0.05$).

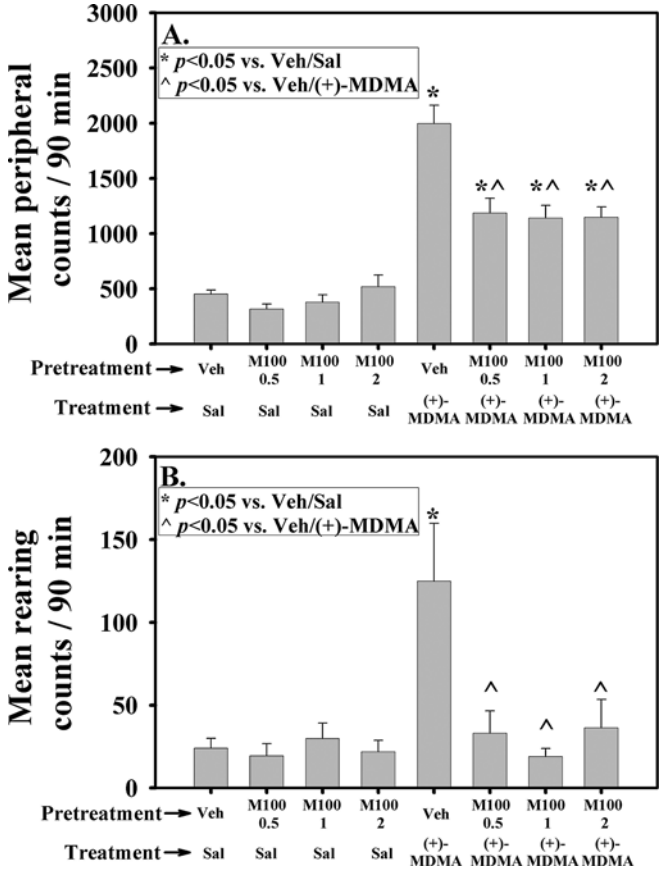

Fig. 1 Effects of M100907 (0, 0.5, 1, and 2 mg/kg) pretreatment on (+)-MDMA-induced (3 mg/kg) peripheral activity and rearing. **a** Mean total peripheral activity (counts per 90 min; ±SEM) in rats ($n=10$) following pretreatment with vehicle (*Veh*; 1 ml/kg, IP) or M100907 (*M100*; 0.5, 1, and 2 mg/kg, IP) and treatment with saline (*Sal*; 1 ml/kg, SC) or (+)-MDMA (3 mg/kg, SC). **b** Mean total rearing (counts per 90 min; ±SEM). Same symbols as in **a**. *Activity counts that were significantly different than vehicle-saline controls ($P<0.05$); ^activity counts that were significantly different than vehicle-(+)-MDMA controls ($P<0.05$)

Effects of M100907 (0, 0.25, 0.5, and 1 mg/kg) pretreatment on the dose-effect curve for (+)-MDMA (0–12 mg/kg) to induce peripheral hyperactivity, rearing and hyperthermia

A main effect of M100907 (0 and 1 mg/kg) pretreatment [$F(1,127)=11.4$, $P=0.001$] and (+)-MDMA treatment [$F(4,127)=138.82$, $P<0.0001$] were observed for peripheral activity. A dose-dependent increase in peripheral locomotor activity was induced by (+)-MDMA (Fig. 2a; $P<0.05$). The (+)-MDMA dose–response curve was shifted to the right after M100907 and the shift was parallel as indicated by the lack of a significant interaction between M100907 pretreatment and (+)-MDMA dose [$F(4,127)=1.72$, $P=0.1499$]. A priori comparisons indicated that that M100907 (1 mg/kg) significantly attenuated hyperactivity evoked by 8 mg/kg and 12 mg/kg (+)-MDMA ($P<0.05$). M100907 alone had no effect on basal peripheral activity ($P>0.05$).

In the absence of a main effect of M100907 (0 and 1 mg/kg) pretreatment [$F(1,127)=0.17$, $P=0.6811$], a main effect of (+)-MDMA treatment [$F(4,127)=18.61$, $P<0.0001$] and a pretreatment×treatment interaction [$F(4,127)=6.06$, $P=0.0002$] were observed for total rearing summed across the 90-min test. (+)-MDMA alone produced an inverted-U-shaped increase in rearing (Fig. 2b; $P<0.05$), with the greatest level of rearing observed at a dose of 8 mg/kg. In the case of (+)-MDMA-induced rearing, the extent of the effects of M100907 were dependent upon the dose of (+)-MDMA tested. A priori comparisons indicated that M100907 (1 mg/kg) significantly suppressed rearing evoked by 8 mg/kg (+)-MDMA ($P<0.05$). Rearing induced by 12 mg/kg (+)-MDMA was significantly increased following M100907 pretreatment ($P<0.05$). M100907 alone had no effect on basal rearing ($P>0.05$).

A main effect of M100907 (0 and 1 mg/kg) pretreatment [$F(1,127)=73.78$, $P<0.0001$] and (+)-MDMA treatment [$F(4,127)=15.6$, $P<0.0001$], and a pretreatment×treatment interaction [$F(4,127)=17.6$, $P<0.0001$] were observed for temperature recorded immediately following behavioral testing. As shown in Fig. 2c, 8 mg/kg and 12 mg/kg (+)-MDMA significantly increased the rectal temperature of experimental animals (~2.5°C increase). A priori comparisons demonstrated that pretreatment with 1 mg/kg M100907 completely prevented the hyperthermic response elicited by (+)-MDMA, reducing rectal temperatures to those of saline-treated animals ($P<0.05$). M100907 alone did not alter basal temperature ($P>0.05$).

The ability of lower doses of M100907 (0, 0.25, and 0.5 mg/kg) to alter (+)-MDMA-induced hypermotility and hyperthermia were also assessed. A main effect of M100907 pretreatment [$F(2,128)=20.45$, $P<0.0001$] and (+)-MDMA treatment [$F(4,128)=127.16$, $P<0.0001$], and a pretreatment×treatment interaction [$F(8,128)=2.89$, $P=0.0057$] were observed for total peripheral activity summed across the 90-min test. (+)-MDMA dose-dependently increased peripheral activity (Fig. 3a; $P<0.05$). Pretreatment with 0.25 mg/kg and 0.5 mg/kg M100907 did not alter basal activity ($P>0.05$), but did reduce peripheral activity produced by either 8 mg/kg or 12 mg/kg (+)-MDMA ($P<0.05$).

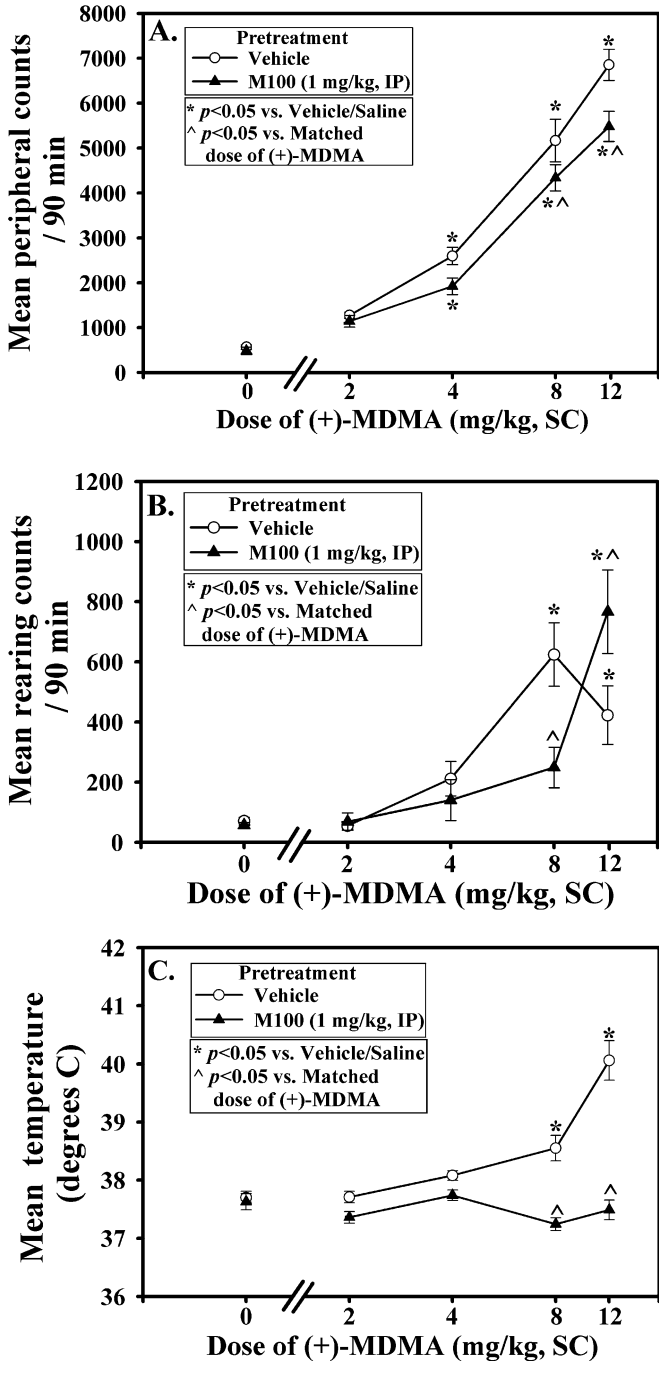

Fig. 2 Effects of M100907 (0 and 1 mg/kg) pretreatment on (+)-MDMA-induced (2, 4, 8, and 12 mg/kg) peripheral activity, rearing, and hyperthermia. **a** Mean total peripheral activity (counts per 90 min; ±SEM) in rats (n=8–16) following pretreatment with vehicle (1 ml/kg, IP) or M100907 (*M100*; 1 mg/kg, IP) and treatment with saline ("0 dose"; 1 ml/kg, SC) or (+)-MDMA (2, 4, 8, and 12 mg/kg, SC). **b** Mean total rearing (counts per 90 min; ±SEM). Symbols as in **a**. **c** Mean rectal temperature (°C; ±SEM) in rats (n=8–16) following behavioral testing. Symbols as in **a**. *Activity counts or temperatures that were significantly different than vehicle-saline controls (P<0.05); ^activity counts or temperatures that were significantly different than vehicle-pretreated animals administered the same dose of (+)-MDMA (P<0.05). Each experimental group contained 12 animals except vehicle/saline (n=16), vehicle/MDMA (8 mg/kg; n=16), M100907/saline (n=8), M100907/MDMA (8 mg/kg; n=16)

Additionally, 0.5 mg/kg M100907 blocked hyperactivity evoked by 4 mg/kg (+)-MDMA (P<0.05) and animals given this combination of treatments exhibited activity that was not different from control animals.

In the absence of a main effect of M100907 pretreatment [F(2,128)=2.77, P=0.0672], a main effect of (+)-MDMA treatment [F(4,128)=37.6, P<0.0001] and a pretreatment× treatment interaction [F(8,128)=7.19, P<0.0001] were observed for rearing summed across the 90-min test, demonstrating that the effects of M100907 on (+)-MDMA-evoked rearing depended upon the dose of (+)-MDMA evaluated. Animals administered vehicle plus saline had low levels of rearing (Fig. 3b) and M100907 did not significantly alter this response (P>0.05). (+)-MDMA dose-dependently increased rearing in a pattern similar to that shown in Fig. 2b, with the greatest level of rearing observed at 8 mg/kg (+)-MDMA, with reduced rearing seen at 12 mg/kg (+)-MDMA (Fig. 3b; P<0.05). Pretreatment with either 0.25 mg/kg or 0.5 mg/kg M100907 significantly suppressed (+)-MDMA-induced rearing at 8 mg/kg (P<0.05), as shown by a priori comparisons. In contrast, both 0.25 mg/kg and 0.5 mg/kg M100907 significantly enhanced rearing evoked by 12 mg/kg (+)-MDMA (P<0.05).

A main effect of M100907 pretreatment [F(2,128)= 47.47, P<0.0001], a main effect of (+)-MDMA treatment [F(4,128)=4.27, P=0.0029], and a pretreatment×treatment interaction [F(8,128)=17.90, P<0.0001] were observed for temperatures assessed following behavioral testing. Treatment with 8 mg/kg or 12 mg/kg (+)-MDMA evoked a significant increase (~2.5°C) in rectal temperature (Fig. 3c; P<0.05). Interestingly, both 0.25 mg/kg and 0.5 mg/kg M100907 had no effect on basal rectal temperature (P>0.05), but completely blocked hyperthermia induced by (+)-MDMA (8 and 12 mg/kg; P<0.05) to an equal degree, reducing the temperature of these animals to that of control animals.

Ability of M100907 (0 and 0.5 mg/kg) to reverse hyperthermia produced by a high dose of (+)-MDMA (12 mg/kg)

A main effect of (+)-MDMA administration [F(1,543)= 60.08, P<0.0001] and M100907 challenge [F(1,543)= 46.80, P<0.0001], time [F(16,543)=13.02, P<0.0001], and a pretreatment×treatment×time interaction [F(16,543)= 14.92, P<0.0001] were observed for temperatures measured across the 4-h test period. Animals pretreated with 12 mg/kg (+)-MDMA alone exhibited a significant increase in rectal temperature across the entire 4-h measurement period (Fig. 4; P<0.05). Subsequent injection of M100907 (0.5 mg/kg) rapidly and completely reversed this response to control levels at every time point except at 135 min, a time at which the temperature of animals treated with the combination of (+)-MDMA and M100907 was actually significantly lower than control levels (P< 0.05); M100907 alone did not alter basal temperature (P>0.05).

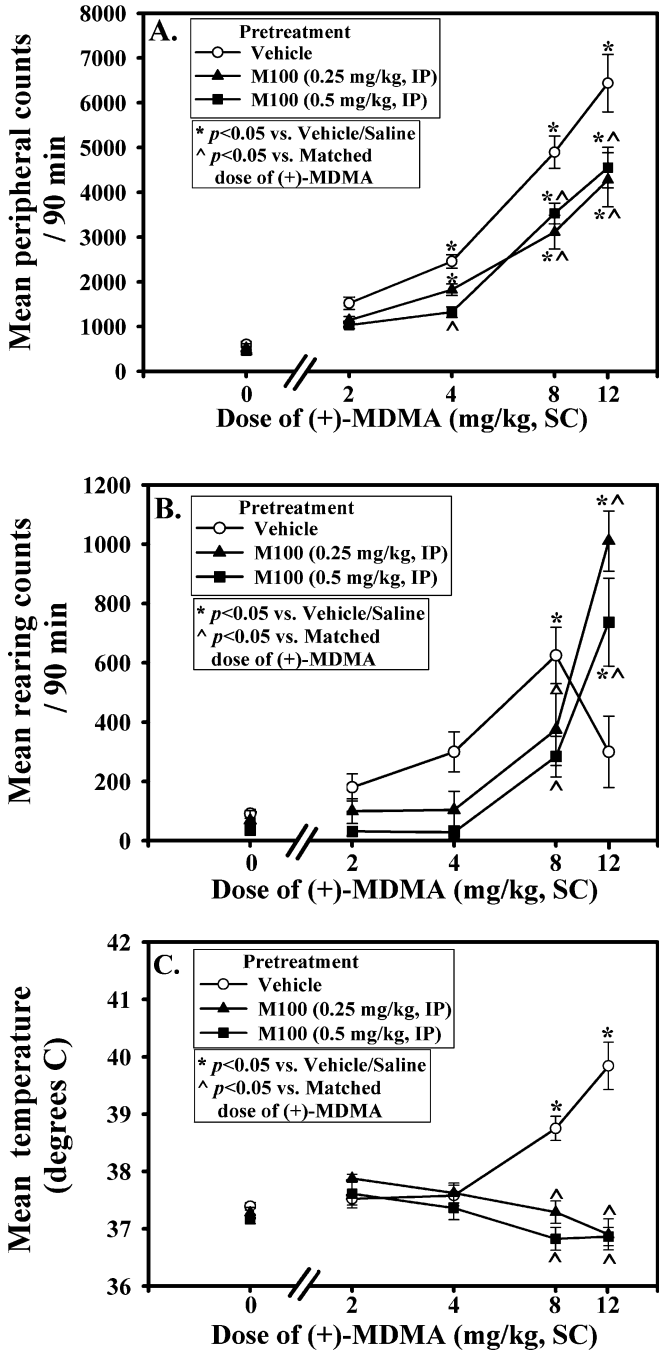

Fig. 3 Effects of M100907 (0, 0.25, and 0.5 mg/kg) pretreatment on (+)-MDMA-induced (2, 4, 8, and 12 mg/kg) peripheral activity, rearing, and hyperthermia. **a** Mean total peripheral activity (counts per 90 min; ±SEM) in rats (n=7–13) following pretreatment with vehicle (1 ml/kg, IP) or M100907 (*M100*; 0.25 and 0.5 mg/kg, IP) and treatment with saline ("0 dose"; 1 ml/kg, SC) or (+)-MDMA (2, 4, 8, and 12 mg/kg, SC). **b** Mean total rearing (counts per 90 min; ±SEM). Symbols as in **a**. **c** Mean rectal temperature (°C; ±SEM) in rats (n=7–13) following behavioral testing. Symbols as in *a*. *Activity counts or temperatures that were significantly different than vehicle-saline controls (P<0.05); ^activity counts or temperatures that were significantly different than vehicle-pretreated animals administered the same dose of (+)-MDMA (P<0.05). Each group contained 8–9 animals except for groups administered vehicle/saline (n=13) and those given vehicle/MDMA (12 mg/kg; n=7)

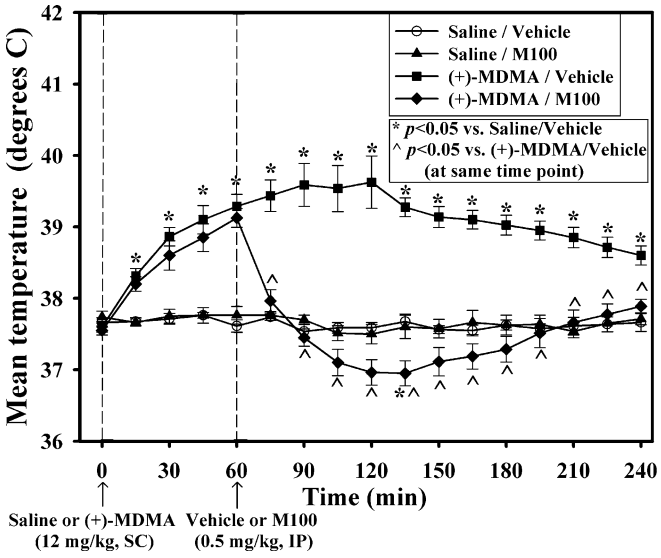

Fig. 4 Ability of M100907 (0 and 0.5 mg/kg) to reverse (+)-MDMA-induced (12 mg/kg) hyperthermia. Mean rectal temperature (°C; ±SEM) in rats (n=8) pretreated with saline (1 ml/kg, SC) or (+)-MDMA (12 mg/kg, SC) and treated with vehicle (1 ml/kg, IP) or M100907 (*M100*; 0.5 mg/kg, IP). *Temperatures that were significantly different than saline-vehicle controls at same time point (P<0.05); ^temperatures that were significantly different than (+)-MDMA-vehicle group at same time point (P<0.05)

Discussion

The present studies indicate the involvement of the 5-HT_{2A}R in the hyperlocomotive and hyperthermic effects of the substituted amphetamine (+)-MDMA. We found that pretreatment with the selective 5-HT_{2A}R antagonist M100907 attenuated peripheral hyperactivity and rearing produced by (+)-MDMA, and completely prevented and reversed hyperthermia produced by (+)-MDMA. The effective doses of M100907 did not alter basal activity or temperature.

These effects of M100907 are most likely due to 5-HT_{2A}R antagonism, given its high affinity (K_i=0.85 nM) for 5-HT_{2A}R; M100907 is selective with low affinity for all other receptors, including the 5-HT_{2B}R, 5-HT_{2C}R, α1-adrenergic receptor, and sigma receptor (K_i=261, 88, 128, and 87 nM, respectively; Kehne et al. 1996a; Roth et al. 2000). Additionally, the ability of M100907 to block behaviors generated by stimulation of 5-HT_{2A}R in Sprague–Dawley rats supports the specificity of the range of doses used here (Hitchcock et al. 1997; Wettstein et al. 1999; McCreary et al. 2003). However, M100907 does lose selectivity for the 5-HT_{2A}R at higher doses (≧3 mg/kg; Patel et al. 2001; Bonaccorso et al. 2002). These data support the range of doses utilized in the present study and suggest that our results are due to blockade of the 5-HT_{2A}R.

Both (+)-MDMA and (±)-MDMA are well characterized to generate horizontal hyperactivity in the periphery of the activity monitor (Paulus and Geyer 1992; McCreary et al. 1999). We demonstrate that the effectiveness of M100907 in blocking (+)-MDMA-induced horizontal hy-

peractivity is dependent upon the dose of (+)-MDMA under study, an observation which explains some discrepancies in the literature. Low doses (3 mg/kg or 4 mg/kg) of (+)-MDMA generate peripheral hyperactivity somewhat resistant to 5-HT_{2A}R blockade (present results; Bankson and Cunningham 2002). In contrast, the 5-HT_{1B}R antagonist GR 127935 completely blocked hyperactivity evoked by a low dose (3 mg/kg) of (+)-MDMA (McCreary et al. 1999). The diverging ability of the 5-HT_{2A}R and 5-HT_{1B}R antagonists to attenuate (+)-MDMA-induced hyperactivity suggests a more dominant role for the 5-HT_{1B}R over 5-HT_{2A}R in hyperactivity generated at 3 mg/kg or 4 mg/kg (+)-MDMA. Serotonin released consequent to (+)-MDMA administration at low doses may interact preferentially at the 5-HT_{1B}R which has a very high affinity for 5-HT (Roth et al. 2000). The superior ability of the 5-HT_{2A}R antagonist to alter the expression of hyperactivity evoked by higher doses of (+)-MDMA (8 and 12 mg/kg; present results) and (±)-MDMA (Kehne et al. 1996b) may be related to a greater indirect stimulation of 5-HT_{2A}R under conditions of greater 5-HT efflux, like that produced by higher doses of (±)-MDMA (Kankaanpaa et al. 1998); 5-HT has a modest affinity for the 5-HT_{2A}R (Peroutka 1986; Roth et al. 2000; Rothman et al. 2000) and stimulation of these receptors may occur predominantly following greater levels of 5-HT release. Additionally, higher doses of (+)-MDMA may directly stimulate 5-HT_{2A}R to evoke hyperactivity given its affinity for this receptor (Battaglia et al. 1988; Nash et al. 1994). This is a possibility, as 5-HT_{2A}R stimulation has been implicated in mediating the hyperactivity induced by such non-selective 5-HT_{2A}R agonists as D-lysergic acid diethylamide (LSD; Ouagazzal et al. 2001) or (±)-1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI; Hillegaart et al. 1996). For example, the hyperactivity induced by low doses of DOI (and seen largely in the periphery of the activity monitor; Bankson and Cunningham, unpublished observations; Hillegaart et al. 1996) or by LSD (Ouagazzal et al. 2001) is blocked by pretreatment with a 5-HT_2R antagonist. In particular, LSD-induced hyperactivity in rats habituated to the activity monitors was completely blocked by pretreatment with M100907 (Ouagazzal et al. 2001). Taken together, these data do support a role for activation of 5-HT_{2A}R in the production of (+)-MDMA-evoked hyperactivity. However, based upon the involvement of the 5-HT_{1B}R (McCreary et al. 1999) and also the DA D_1R and D_2R (Gold et al. 1989; Kehne et al. 1996b; Bubar et al. 2004), the 5-HT_{2A}R is a component in a complex interplay of neural systems that underlie the behavioral and physiological effects of MDMA.

A recent study in mice showed that M100907 *enhanced* horizontal hyperactivity elicited by 32 mg/kg (+)-MDMA (Fantegrossi et al. 2003). High doses of (±)-MDMA evoke behaviors characteristic of the 5-HT syndrome, including flat body posture (unpublished observations; Spanos and Yamamoto 1989). At the doses of (+)-MDMA utilized here (2–12 mg/kg), the expression of flat body posture did not correlate with reduced horizontal activity evoked by (+)-MDMA in rats, since animals still exhibit horizontal hyperactivity regardless of body posture (unpublished observations). However, the magnitude of 5-HT syndrome behaviors elicited by 32 mg/kg (+)-MDMA in mice could limit full expression of horizontal hyperactivity; inhibition of this behavioral syndrome by M100907 might enhance horizontal activity. This possibility is supported by reports indicating a role for the 5-HT_{2A}R in components of the 5-HT syndrome (Nisijima et al. 2001; Van Oekelen et al. 2002).

The generation of rearing by MDMA has not been well characterized and we provide the first evidence that the 5-HT_{2A}R controls rearing evoked by (+)-MDMA. The 3 mg/kg dose of (+)-MDMA appears to be a threshold dose for the induction of rearing, as this dose has been shown to increase rearing or have no effect under identical test conditions (McCreary et al. 1999; Bankson and Cunningham 2002). In the present studies, (+)-MDMA alone produced an inverted-U-shaped dose–response curve for rearing, with the maximal level of rearing seen at 8 mg/kg. M100907 pretreatment shifted this dose-effect curve to the right, without altering basal rearing, and attenuated rearing elicited by doses of (+)-MDMA as high as 8 mg/kg, suggesting that activation of the 5-HT_{2A}R subsequent to (+)-MDMA administration results in the production of rearing. However, the frequency of rearing produced by 12 mg/kg (+)-MDMA alone was reduced below that observed at 8 mg/kg. At this dose (12 mg/kg), 5-HT syndrome behaviors, especially flat body posture (unpublished observations), may compete with the expression of rearing. An attenuation of some components of the 5-HT syndrome by 5-HT_{2A}R blockade, particularly flattened body posture, might be expected to enhance rearing at 12 mg/kg (+)-MDMA.

The mechanisms underlying the attenuation of (+)-MDMA-induced hyperactivity with M100907 potentially involve a 5-HT_{2A}R modulation of the mesoaccumbens DA system, which contains DA cell bodies in the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc). This pathway plays a key role in the generation of stimulant-induced behaviors (Delfs et al. 1990) and appears to mediate the locomotor stimulant effects of (±)-MDMA (Gold et al. 1989). We postulate that 5-HT released consequent to (+)-MDMA administration stimulates the 5-HT_{2A}R on DA neurons (Doherty and Pickel 2000) to enhance their firing (Pessia et al. 1994) and result in increased DA efflux in the NAc (Schmidt et al. 1992). A second possible mechanism for 5-HT_{2A}R antagonism to alter (+)-MDMA-evoked hyperactivity may involve blockade of DA synthesis by M100907, resulting in decreased DA release in terminal regions (Schmidt et al. 1992). Previous studies have suggested that activation of 5-HT_{2A}R may be necessary for enhanced DA synthesis under conditions of stimulated DA neurotransmission, like that produced by (±)-MDMA (Schmidt et al. 1992). Thus, antagonism of 5-HT_{2A}R by M100907 may interrupt the (+)-MDMA-evoked enhancement of the DA system and suppress (+)-MDMA-evoked hyperactivity, a hypothesis supported by a recent study demonstrating that M100907 decreased hyperactivity produced by elevated synaptic

levels of DA in DA transporter knockout mice (Barr et al. 2004).

The more potent isomer (+)-MDMA dose-dependently elevated rectal temperature and pretreatment with M100907 blocked this response. This finding is in agreement with studies in which M100907 attenuated hyperthermia elicited by (±)-MDMA in rats and mice (Mechan et al. 2002; Fantegrossi et al. 2003). However, our data conflict with one study in which M100907 did not alter hyperthermia induced by the (+)-isomer of MDMA in mice (Fantegrossi et al. 2003), suggesting that the contribution of the 5-HT_{2A}R to hyperthermia elicited by the more potent isomer (+)-MDMA may depend on the species or strain of animal. Interestingly, the magnitude of the blockade of (+)-MDMA-induced hyperthermia following administration of M100907 greatly exceeds the blockade of peripheral hyperactivity or rearing evoked by (+)-MDMA. This dissociation between hyperthermia and motor activity suggests that the generation of hyperthermia is independent of motor activity and that the underlying mechanisms overlap but are not identical.

The present study is the first to investigate reversal of (+)-MDMA-evoked hyperthermia by a selective 5-HT_{2A}R antagonist. We found that hyperthermia evoked by a high dose (12 mg/kg) of (+)-MDMA was rapidly and completely reversed by M100907, suggesting that this physiological consequence of (+)-MDMA administration is largely dependent upon activation of the 5-HT_{2A}R. Interestingly, the antipsychotic clozapine was shown to reverse hyperthermia evoked by (±)-MDMA in rats and rabbits, an effect attributed to either antagonism of 5-HT_{2A}R or activation of 5-HT_{1A}R (Blessing et al. 2003). Our findings suggest that the reversal of (±)-MDMA-induced hyperthermia induced by clozapine is due to 5-HT_{2A}R antagonism.

Collectively, our results suggest a possible therapeutic role for selective 5-HT_{2A}R antagonists in the treatment of the behavioral, psychological, and physiological effects of MDMA in human users of the drug. The 5-HT_2R antagonist ketanserin was shown to attenuate emotional excitation, perceptual changes, and increased temperature evoked by (±)-MDMA in humans, supporting the possible clinical utility for 5-HT_{2A}R antagonists in the treatment of these effects in human (±)-MDMA users (Liechti et al. 2000). Treatment of (±)-MDMA-induced hyperthermia is of particular importance, as it can lead to life-threatening medical complications (Dar and McBrien 1996). Animal studies suggest that hyperthermia exacerbates (±)-MDMA-evoked 5-HT neurotoxicity (Malberg and Seiden 1998), which may produce long-term psychological effects (Morgan 2000). Current treatments for hyperthermia in humans include administration of the muscle relaxant dantrolene and/or ice baths (Dar and McBrien 1996). While both treatments do reverse hyperthermia, neither of them is specific for direct blockade at the key central sites of action for (±)-MDMA, which likely contribute to the generation of hyperthermia (Blessing et al. 2003). M100907, however, completely prevented hyperthermia produced by (+)-MDMA, most likely through a direct action at 5-HT_{2A}R. Thus, by attenuating the subjective (Liechti et al. 2000) and hyperthermic effects of (±)-MDMA, M100907 or other selective 5-HT_{2A}R antagonists may be effective in reducing the medical and psychological consequences of (±)-MDMA use.

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