3,4-Methylenedioxymethamphetamine- and 8-Hydroxy-2-di-n-propylamino-tetralin-Induced Hypothermia: Role and Location of 5-Hydroxytryptamine 1A Receptors

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ABSTRACT

The popular drug of abuse 3,4-methylenedioxymethamphetamine (MDMA) has complex interactions with thermoregulatory systems, resulting in either hyperthermia or hypothermia. MDMA induces hypothermia when given to animals housed at a low ambient temperature. In this study we report that MDMA (7.5 mg/kg i.p.) given at normal ambient temperatures of 24 to 25°C caused, in conscious freely moving rats, hypothermia (mean decrease from baseline of 1.1 ± 0.06°C at 40 min). Pretreating animals with a 0.5 mg/kg i.p. dose of the 5-hydroxytryptamine 1A (5-HT1A) antagonist [2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100635) not only prevented MDMA-induced hypothermia, but resulted in the development of hyperthermia (mean temperature increase from baseline of 0.74 ± 0.2°C at 120 min). After treatment with WAY 100635, MDMA also elicited an enhanced tachycardia (mean increases in heart rate from baseline of 110 ± 16 beats/min at 90 min). To identify the location of 5-HT1A receptors responsible for hypothermia induced by MDMA, we first investigated the role of 5-HT1A receptors in the rostral raphe pallidus (rRP) in decreases in temperature evoked by the known 5-HT1A agonist 8-hydroxy-2-di-n-propylamino-tetralin (DPAT). Microinjections of 0.5 nmol of WAY 100635 into the rRP significantly attenuated DPAT (0.2 mg/kg i.p.)-elicited hypothermia. In parallel experiments, we found that microinjections of WAY 100635 into the rRP, while significantly augmenting MDMA-mediated tachycardia, did not alter body temperature. These results demonstrate that although hypothermia mediated by both MDMA and DPAT shares a common dependence on the activation of 5-HT1A receptors, the location of these receptors is different for each drug.

Persons using substituted amphetamines such as 3,4-methylenedioxymethamphetamine (MDMA) may, by mechanisms not completely understood, develop severe hyperthermia and cardiovascular collapse (Gowing et al., 2002). Although hyperthermia remains the best studied physiologic effect related to MDMA usage, hypothermia can also occur, a phenomenon that is often ignored or, when acknowledged, is attributed to low ambient temperatures (Malberg and Seiden, 1998). MDMA, however, can evoke decreases in body temperature at normal or even elevated ambient tempera-

tures (Malberg et al., 1996; Bexis and Docherty, 2006). Even in studies in which MDMA causes hyperthermia, a transient initial period of hypothermia or a delay in the development of hyperthermia is often seen (Gordon et al., 1991; Malberg et al., 1996). To date few studies have addressed the mechanisms responsible for MDMA-induced hypothermia.

The principal pharmacologic action of MDMA is facilitation of transmission at monoaminergic nerve terminals, causing the release of monoamines in the central nervous system and more specifically elevating extracellular levels of serotonin, dopamine, and norepinephrine (Rothman et al., 2001; Gough et al., 2002; Sprague et al., 2007), all of which have been implicated in hyperthermia induced by MDMA. The mechanisms behind hypothermia induced by MDMA are, however, largely unknown. Serotonin can elicit opposite effects on body temperature, depending on whether it binds to serotonergic 1A (5-HT1A) or 2A (5-HT2A) receptors. The divergent effects of serotonin on body temperature present an attractive hy-
pothesis for changes in temperature induced by MDMA. To date, the role of serotonin in body temperature changes evoked by MDMA has focused on 5-HT$_{2A}$ receptors with 5-HT$_{2A}$ antagonists reducing hyperthermia and neurotoxicity elicited by MDMA (Schmidt et al., 1990). 5-HT$_{2A}$ receptors are involved in hyperthermia evoked by MDMA by impairing heat dissipation through the constriction of cutaneous vascular beds (Blessing et al., 2003; Ootsuka et al., 2004) and by increasing heat generation through intercapider brown adipose tissue (IBAT) (Blessing et al., 2006). Whereas 5-HT$_{2A}$ receptors contribute to MDMA-mediated hyperthermia, the role of 5-HT$_{1A}$ receptors in temperature effects elicited by MDMA is largely unknown. As serotonin has a >1000-fold affinity for 5-HT$_{1A}$ receptors over that of 5-HT$_{2A}$ receptors (Peroutka et al., 1981), its release by MDMA probably activates 5-HT$_{1A}$ receptors. Activation of 5-HT$_{1A}$ receptors by agonists such as 8-hydroxy-2-di-n-propylamino-tetralin (DPAT) causes, similar to MDMA, the serotonin behavioral syndrome (Goodwin et al., 1987) and in a manner opposite to 5-HT$_{2A}$ receptor activation, hyperthermia (Gudelsky et al., 1986; Ootsuka and Blessing, 2003). Along with their individual contributions, 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors have complex interactions affecting body temperature. Hyperthermia elicited by stimulating 5-HT$_{2A}$ receptors with (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-1-propane hydrochloride is potentiated by the 5-HT$_{1A}$ antagonist WAY 100635 and prevented by the 5-HT$_{1A}$ agonist DPAT (Salmi and Ahlenius, 1998). Similarly, WAY 100635 augments the hyperthermia induced by MDMA in a warm environment (Saadat et al., 2005). Together these data suggest that the overall effect of MDMA on body temperature may reflect the net sum of the combined activities of serotonin on 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors.

Muscle activity represents a key component of thermoregulation in the rat in the form of shivering and work-related motor activity. A direct relationship exists between work intensity and core body temperature (Gordon, 1990). MDMA increases locomotion in rats, a factor that probably contributes to the development of hyperthermia (Spanos and Yamamoto, 1989). Locomotion by itself however, is insufficient to explain the effects of MDMA on body temperature as the degree of locomotion induced by a dose of MDMA is similar regardless of whether the animal develops hypo- or hyperthermia, depending on the ambient temperature in which it is given (Dufters, 1994). The role 5-HT$_{1A}$ receptors play in the locomotor effects induced by MDMA is unclear as 5-HT$_{1A}$ antagonists have been reported to both cause reduction and have no effect on MDMA-evoked locomotion (Calloway et al., 1992; McCready et al., 1999).

The cardiovascular system plays a critical role in normal thermoregulation. Activation of the sympathetic nervous system in response to a cold environment results in the constriction of cutaneous vessels, along with an increase in heart rate and cardiac output (Blessing, 2004; Deussen, 2007). The net result of these effects is to conserve heat by shunting blood away from a cool skin surface while simultaneously supporting heat generation by diverting blood to heat-producing central organs and brown adipose tissue. The reverse of these effects occurs in a warm environment, facilitating heat release (Ootsuka and Blessing, 2006b). Whereas the effects of MDMA on cutaneous blood flow have been well studied (Pedersen and Blessing, 2001), its effects on other components of the cardiovascular system are less well known. Although it causes tachycardia in humans (Lester et al., 2000), the cardiovascular effects of MDMA in rats have not been clearly delineated, as both an increase (Gordon et al., 1991) and no effect on heart rate have been reported (Irvine et al., 2001; Bexis and Docherty, 2006).

The rostral raphe pallidus (rRP) is a serotonin-rich region of the medulla containing sympathetic thermoregulatory premotor neurons that control the sympathetic innervation of cutaneous blood vessels and IBAT in the rat (DiMicco et al., 2006). Neurons in the rRP are known to express 5-HT$_{1A}$ receptors (Helke et al., 1997), and studies to date have demonstrated that microinjections of DPAT into the rRP block thermogenesis induced by leptin and cutaneous vasoconstriction induced by cold (Morrison, 2004; Ootsuka and Blessing, 2006a). Likewise microinjections of DPAT into various regions of the brainstem and rRP inhibit the cardioexcitatory responses to leptin and to hypothalamic stimulation (Morrison, 2004; Horiiuchi et al., 2005).

The cardiovascular and thermogenic responses to various stressors share similar hypothalamic and brainstem mechanisms (DiMicco et al., 2006). Thus, we sought to investigate the role of 5-HT$_{1A}$ receptors in the region of the rRP of conscious, freely moving rats in 1) the thermoregulatory, cardiovascular, and locomotor effects induced by MDMA and 2) the hypothermia induced by systemic administration of DPAT.

Materials and Methods

All procedures conformed to the guidelines set forth by the National Institutes of Health and were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee.

Drugs

For systemic administration, WAY 100635 (Sigma-Aldrich, St. Louis, MO) was dissolved to concentration with normal saline and used on the day of the experiment. For microinjections, WAY 100635 was dissolved in 0.1 M phosphate-buffered saline (PBS) and used on the same day or frozen at −80°C and used within 2 weeks. DPAT (Sigma-Aldrich) was dissolved in normal saline and used on the day of the experiment. MDMA was generously provided by the National Institutes of Health and prepared at the time of the experiments in normal saline.

Animals

Male Sprague-Dawley rats (325 ± 25 g; Harlan, Indianapolis, IN) were maintained in a 12-h light/dark cycle beginning at 7 A.M. and fed ad libitum. Animals were housed singly to avoid interference from competing radiotelemetric signals and to avoid potential chewing or damage to central nervous system guide cannulas from other animals.

Surgical Preparation

For studies using MDMA and WAY 100635, rats were anesthetized (80 mg/kg ketamine and 11.5 mg/kg xylazine i.p., supplemented as required) and a telemetric transmitter (model CS0-PXT; Data Sciences, St. Paul, MN) was implanted as described previously for the measurement of mean arterial pressure, heart rate, locomotion, and core body temperature (Zaretsky et al., 2003). For studies with DPAT and those to determine the effects of different doses of MDMA on body temperature, a telemetric transmitter measuring only core temperature and locomotion (model TX-P40; Data Sciences) was implanted into the abdominal cavity.
For experiments involving microinjections into the rRP, rats were anesthetized 5 days after transmitter implantation and placed in a stereotaxic apparatus with the incisor bar set 3.3 mm below the interaural line for placement of a guide cannula as described previously (Zaretsky et al., 2003). The target coordinates were anterior-posterior −2.8 mm, left-right 0.0 mm, and height-depth −1.1 mm with the interaural line as a reference point. Dummy wire cannulas were inserted in the guides, and rats were returned to their cages and left undisturbed until they regained their approximate presurgery weights (approximately 5–7 days).

Experiments
Experiments were conducted in the animal’s home cage in quiet and isolated rooms between 10:00 A.M. and 2:00 P.M. Animals acclimated to their new environment for a minimum of 2 h before experiments were initiated. Throughout the experiment ambient temperature was maintained at 24 to 25°C, a temperature typically associated with MDMA-induced hyperthermia (Malberg and Seiden, 1998). In all the experiments, animals were randomized to study groups in blocks of eight using a random number generator.

Dose Response. To determine the dose causing hypothermia and the time of maximal effect, rats received an i.p. dose of either saline or one of three doses of MDMA (1.5, 7.5, or 15 mg/kg). We recorded temperature in degrees Celsius and locomotion in units of activity every minute for 120 min after vehicle or MDMA.

Effect of Systemic Treatment with WAY 100635 on MDMA-Induced Hypothermia. Animals were assigned to one of four groups (saline/saline, WAY/saline, saline/MDMA, or WAY/MDMA; n = 8 rats/group). After an acclimation period, each animal received a 0.3-ml i.p. injection of either saline vehicle (0.9%) or WAY 100635 (0.5 mg/kg) followed 5 min later by an equal volume of either saline or MDMA (7.5 mg/kg). Temperature (degrees Celsius), locomotion (units of activity), heart rate in beats per minute, and mean arterial pressure in millimeters of mercury were recorded every minute for 120 min after MDMA injection. Animals originally receiving i.p. injections of saline received microinjections of either PBS or WAY 100635 in a crossover fashion. To avoid the possibility of MDMA-induced serotonin neurotoxicity confounding the effect on body temperature, animals receiving MDMA were not crossed over. Animals were microinjected into the region of the rRP with 100 nl of PBS (0.1 M) or WAY 100635 (5 mM solution; 0.5 nmol) over 30 s depending on group assignment. Five minutes later animals received an i.p. injection of 0.3 ml of either saline vehicle or MDMA (7.5 mg/kg). We recorded temperature (degrees Celsius), locomotion (units of activity), heart rate (beats per minute), and mean arterial pressure (millimeters of mercury) every minute for 120 min after MDMA injection. Injection sites were verified using fluorescent microspheres as described above.

Data Analysis. Data were captured using a receiver placed under the rat’s cage and transmitted to an IBM-compatible computer using the Dataquest A.R.T data acquisition system (Data Sciences). Statistics were run using SPSS 14.0 for Windows (SPSS, Chicago, IL). Graphs were made using Prism 4.0 (GraphPad Software, San Diego, CA) software. Grouped data are reported as 5-min means ± 1 S.E. measurement for the time points shown. To compare changes in temperature and activity from baseline in the dose-response experiment we performed a one-way repeated measures ANOVA with pairwise comparisons done using the LSD procedure. For the remainder of the studies we performed a two-way full factorial repeated measures analysis of variance. We included in the model main effects for study group, time, and their interaction. We performed post hoc comparisons among time points within a study group and between study groups within a time point. Pairwise comparisons were adjusted using a LSD procedure.

RESULTS

Dose Response of MDMA on Temperature and Locomotion

Neither saline nor 1.5 mg/kg MDMA significantly affected temperature or locomotion (Fig. 1). At a dose of 7.5 mg/kg, MDMA evoked a purely hypothermic response with a maximum temperature decrease of 1.1 ± 0.06°C from baseline (37.2 ± 0.1°C) and returning to baseline by 80 min. Conversely, a 15 mg/kg dose of MDMA elicited a purely hyperthermic response with a maximum increase in temperature of 1.6 ± 0.3°C from baseline (37.0 ± 0.08°C) occurring 120 min after injection. Although causing divergent effects on body temperature, doses of 7.5 and 15 mg/kg MDMA evoked similar increases in motor activity.
were evident with 15 mg/kg MDMA. Dashed horizontal lines indicate times at which significant differences were found by ANOVA and LSD analysis, which there were significant differences from baseline (by repeated measures ANOVA and LSD analysis, p < 0.05) in rats treated with 7.5 mg/kg. Solid horizontal bars indicate times in which significant differences were evident with 15 mg/kg MDMA. n = 6 for each group. au, units of activity.

**Effect of Systemic Treatment with WAY 100635 on MDMA-Induced Changes**

There were no differences in baseline values of any measured parameter among any of the treatment groups (Fig. 2).

**Temperature.** Injections of saline had no effect on body temperature (Fig. 2A). Injections of WAY 100635 followed by saline, however, caused an increase in body temperature that by 30 min was significantly different both from its baseline (+0.8 ± 0.2°C, p = 0.002) and from the corresponding 30 min values of all other groups. The effects of WAY 100635 were transient, however, returning to baseline values by 60 min. The injection of saline followed by MDMA (saline/MDMA) caused declines in body temperature at both 30 min (−0.9 ± 0.2°C, p = 0.001) and 60 min (−0.8 ± 0.3°C, p = 0.007) that were significant compared with both its own baseline and with the corresponding values of all other groups. Not only did pretreatment with WAY 100635 prevent MDMA-induced hypothermia, but animals treated with WAY followed by MDMA actually developed hyperthermia with temperatures significantly increasing 0.7 ± 0.2°C from baseline at both 90 and 120 min (p < 0.01 for both).

**Locomotion.** Outside of transient effects, secondary to the stress of the i.p. injections, neither the injection of saline/saline nor that of WAY/saline resulted in significant increases in locomotion (Fig. 2B). Regardless of pretreatment, the injection of MDMA resulted in significant increases in locomotor activity at every time point after its injection. Pretreatment with WAY 100635 attenuated the increases in locomotion induced by MDMA at 30 min (9.8 ± 3 versus 21.2 ± 3 au, p = 0.019), but by 60 min these effects were no longer evident (p = 0.2).

**Heart Rate.** In all the groups, regardless of the pretreatment, the first injection (represented by 0 min) resulted in an increase in heart rate (Fig. 2C). In the saline/saline group no further increases were seen. In those animals injected with WAY/saline, however, a significant increase in heart rate compared with baseline was still evident at 30 min (+51 ± 18 beats/min, p = 0.009). The injection of saline followed by MDMA caused a steady increase in heart rate beginning at 30 min and becoming significantly increased compared with baseline by 90 min (+79 ± 18 beats/min, p < 0.001). In those rats pretreated with WAY 100635, increases in heart rate after the injection of MDMA were immediate and sustained throughout the experiment. At both 30 and 60 min, values for heart rate in the WAY/MDMA group were greater than those of any of the other groups.

**Mean Arterial Pressure.** Similar to heart rate, mean arterial pressure transiently increased after pretreatment injections (represented by 0 min) in each group (Fig. 2D). In only those animals injected with WAY 100635 followed by MDMA, however, were increases in MAP significant compared with baseline at subsequent time points.

**Identification of Microinjection Sites**

Microinjection sites (Fig. 3) were determined using different colored fluorescent microspheres with the corresponding centers of the visualized microspheres plotted on representative drawings adapted from Paxinos and Watson (1998) and shown in Fig. 3D. Shown in Figs. 3, A to C are examples of fluorescent micrographs with the matching counterstained section. We considered positive injections as those in which the center of the fluorescence was contiguous with or within the area of the raphe pallidus.

**Microinjection of WAY 100635: DPAT.** There were no differences in baseline values of any measured parameter among any of the treatment groups (Fig. 4).

**DPAT: Core Body Temperature.** Microinjections of either 0.5 nmol of WAY 100635 (100 nl, 5 mM) or PBS (100 nl) into the region of the rRP followed by the i.p. injection of saline caused a transient increase in core body temperature (saline/saline and WAY/saline) (Fig. 4A). As expected, microinjection of PBS followed by i.p. injection of a 0.2 mg/kg dose of the 5-HT1A agonist DPAT (PBS/DPAT) caused a rapid decline in core body temperature. Temperatures in the PBS/DPAT group were significantly below baseline for the entire postinjection period with the nadir occurring at 30 min (−1.4 ± 0.2°C from baseline, p < 0.001). The rRP was shown to be a key area involved in DPAT-induced hypothermia as microinjections of WAY 100635 (0.5 nmol) in the rRP significantly attenuated the drop in temperature (−0.4 ± 0.3°C, p = 0.004) seen at 30 min in the PBS/DPAT group. If microinjections of WAY 100635 fell outside of the region of the rRP [WAY/DPAT (OR)] reductions in DPAT-evoked hypothermia...
were no longer evident at 30 min as decreases in body temperature (−1.4°C ± 0.3°C) were identical to those seen in the PBS/DPAT group (−1.4 ± 0.2°C, p = 0.6).

**DPAT: Locomotion.** As the locomotor effects elicited by DPAT are short-lived, we added the 15-min time point to the analysis of motor activity. Microinjections of either PBS or 0.5 nmol of WAY 100635 followed by the systemic injection of saline (PBS/DPAT and WAY/DPAT) had no effect on locomotor activity (Fig. 4B). All of the groups given i.p. injections of DPAT (PBS/DPAT and WAY/DPAT) demonstrated significant and similar increases in locomotion 15 min postinjection.

**Microinjection of WAY 100635: MDMA**

There were no differences in baseline values of any measured parameter among any of the treatment groups (Fig. 5).

**MDMA: Core Body Temperature.** Microinjections of PBS followed by the i.p. injection of saline (PBS/saline) increased core body temperature at both 30 and 60 min postinjection (Fig. 5A) in a manner comparable with that seen in the PBS/DPAT group (Fig. 4A) in the previous experiment. Likewise, the microinjection of WAY 100635 followed by i.p. saline (WAY/saline) increased body temperature, although differences from baseline did not achieve significance. Different from the systemic administration of saline or WAY 100635 followed by MDMA (7.5 mg/kg i.p.) (Fig. 2A), microinjections of PBS or WAY 100635 followed by systemic MDMA (PBS/MDMA and WAY/MDMA) did not significantly reduce core body temperature compared with baseline values. Although the core temperature for each MDMA group was significantly different from its respective control at 30 min (37 ± 0.2°C for PBS/MDMA versus 38 ± 0.2°C for PBS/saline, p = 0.002 and 37.1 ± 0.2°C for WAY/MDMA versus 37.8 ± 0.2°C for WAY/saline, p = 0.039), they were not different from one another (p = 0.8).

**MDMA: Locomotion.** Systemic injections of MDMA significantly increased locomotion at all time points after its injection (Fig. 5B). Unlike the systemic administration of WAY 100635 (Fig. 2B), which attenuated MDMA-mediated locomotion, microinjections of WAY 100635 had no affect on locomotion induced by MDMA.
Fig. 3. Photomicrographs of coronal rat brain sections from a representative microinjection experiment. The distribution of red (A) and green (B) fluorescent microspheres marks the sites of injections of 0.5 nmol of WAY 100635 (100 nl, 5 mM) and PBS (100 nl, 0.1 M), respectively. The corresponding neutral red counterstained section (C) shows anatomic landmarks used for marking injection sites. D, schematic diagrams of coronal sections through rat medulla adapted from the atlas of Paxinos and Watson (1998), illustrating approximate sites of injection for all of the described microinjection experiments. Numbers indicate distance from bregma in millimeters. □, injections of WAY 100635 or PBS followed by systemic injection of DPAT; ■, injections of WAY 100635 or PBS outside the region of the rRP followed by the systemic injection of DPAT; ▲, injections of PBS followed by the systemic injections of MDMA; △, WAY 100635 followed by the systemic injections of MDMA; ○, injections of WAY 100635 outside the region of the rRP followed by systemic injections of MDMA. py, pyramids; bas, basilar artery.
EDTA, consistent and sustained elevations in mean arterial pressure did not occur in any of the groups (Fig. 5D). This result is different from that seen after the systemic administration of WAY 100635 followed by MDMA (Fig. 2) for which mean arterial pressure increased in a manner similar to heart rate.

Discussion

This study presents new findings regarding the physiologic changes induced by MDMA and DPAT. Our results indicate that the 5-HT\textsubscript{1A} antagonist WAY 100635 not only prevents hypothermia mediated by MDMA at room temperature but also results in the development of hyperthermia. The effects of WAY 100635 on temperature changes elicited by MDMA are independent of locomotion, as WAY 100635 attenuates early increases in locomotion mediated by MDMA. In a manner similar to its effects on temperature, WAY 100635 unmasks significant MDMA-mediated tachycardia. Analogous to MDMA, systemic administration of DPAT causes hypothermia and for the first time we demonstrate that the 5-HT\textsubscript{1A} receptors responsible for DPAT-induced hypothermia are located, at least in part, in the region of the rRP. The location of 5-HT\textsubscript{1A} receptors involved in suppressing MDMA-mediated tachycardia, but not those in mediating hypothermia, is likewise in the rRP.

A potential confounder in our results was that when administered systemically, WAY 100635 alone increased temperature and heart rate (Fig. 2, A and C). That WAY 100635 alone can increase temperature has been reported previously (Saadat et al., 2005). We do not, however, believe that the differences seen between animals treated with WAY/MDMA and saline/MDMA occurs simply via a competitive physiologic mechanism. First, the degree to which WAY 100635 affects temperature and heart rate cannot by itself account for the changes seen between WAY/MDMA and saline/MDMA animals. Second, the effects WAY 100635 alone had on temperature and heart rate were transient, whereas its effects on changes in temperature and heart rate induced by MDMA were more sustained.

Serotonin has long been implicated in the regulation of body temperature with initial studies showing that its microinjection into the anterior hypothalamus caused hyperthermia (Cox et al., 1980). Contrary to this fact, however, the systemic injection of drugs such as MDMA that increase the central release of serotonin typically causes hypothermia (Gordon et al., 1991). These seemingly conflicting findings may be due to the opposing effects of serotonin on 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors. Agonists of 5-HT\textsubscript{1A} receptors, such as DPAT, cause dose-dependent hypothermia (Gudelsky et al., 1986) by increasing heat loss through the dilation of cutaneous vessels and by decreasing heat generation through inhibition of IBAT (Ootsuka and Blessing, 2006a,b). Activation of 5-HT\textsubscript{2A} receptors on the other hand, causes hyperthermia through cutaneous vasoconstriction and by increasing heat production in brown adipose tissue (Ootsuka et al., 2004; Ootsuka and Blessing, 2006b). Therefore, drugs such as MDMA, which cause a large release of serotonin may affect both 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors. Because serotonin has >1000 times the affinity for 5-HT\textsubscript{1A} receptors than for 5-HT\textsubscript{2A} receptors (Peroutka et al., 1981), a low dose of MDMA might affect 5-HT\textsubscript{1A} receptors to a greater extent, thereby causing hypothermia. Conversely, higher doses of MDMA

**Fig. 4.** Changes in core temperature (A) and locomotion (B) after microinjections (t = -5 min) of WAY 100635 (100 nl, 5 mM) or PBS (100 nl) into the rRP followed by (t = 0 min) by i.p. injections of DPAT (0.2 mg/kg) or saline. Animals in which microinjections of WAY 100635 were outside the rRP are designated by the abbreviation (OR). *p < 0.05, **p < 0.01. [PBS/saline n = 6, WAY 100635/saline n = 5, WAY/DPAT n = 5, WAY/DPAT (OR) n = 7.]

**MDMA: Heart Rate.** As seen with temperature (Fig. 5A), the microinjection procedure caused a stress response in the animals manifested as transient increases in heart rate in both the PBS/saline and WAY/saline groups (Fig. 5C). Microinjections of PBS (100 nl) followed by systemic administration of MDMA (PBS/MDMA) caused a gradual increase in heart rate that was significantly different from baseline by 60 min (+42 ± 17 beats/min, p = 0.02) and from then on. As with the systemic administration of WAY 100635 followed by MDMA (Fig. 2B), microinjections of WAY 100635 (0.5 nmol) into the rRP resulted in significant increases in MDMA-mediated tachycardia at 30 min and from that point on compared with all other groups, except the WAY/MDMA (OR) group in which numbers of animals were too small (n = 4) for comparison. In animals with microinjection cannulae misplaced outside the rRP (OR), microinjections of WAY 100635 failed to alter significantly the effect of systemically administered MDMA on heart rate compared with the PBS/MDMA group.

**MDMA: Mean Arterial Pressure.** Except for the transient increases in mean arterial pressure seen after microinjections, consistent and sustained elevations in mean arterial pressure were not seen in any of the groups. The mean arterial pressure did not differ significantly between the various groups at the specified time point. ANOVA and LSD, p < 0.05. [PBS/MDMA (OR) n = 5, WAY/DPAT (OR) n = 7.]

Serotonin has long been implicated in the regulation of body temperature with initial studies showing that its microinjection into the anterior hypothalamus caused hyperthermia (Cox et al., 1980). Contrary to this fact, however, the systemic injection of drugs such as MDMA that increase the central release of serotonin typically causes hypothermia (Gordon et al., 1991). These seemingly conflicting findings may be due to the opposing effects of serotonin on 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors. Agonists of 5-HT\textsubscript{1A} receptors, such as DPAT, cause dose-dependent hypothermia (Gudelsky et al., 1986) by increasing heat loss through the dilation of cutaneous vessels and by decreasing heat generation through inhibition of IBAT (Ootsuka and Blessing, 2006a,b). Activation of 5-HT\textsubscript{2A} receptors on the other hand, causes hyperthermia through cutaneous vasoconstriction and by increasing heat production in brown adipose tissue (Ootsuka et al., 2004; Ootsuka and Blessing, 2006b). Therefore, drugs such as MDMA, which cause a large release of serotonin may affect both 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors. Because serotonin has >1000 times the affinity for 5-HT\textsubscript{1A} receptors than for 5-HT\textsubscript{2A} receptors (Peroutka et al., 1981), a low dose of MDMA might affect 5-HT\textsubscript{1A} receptors to a greater extent, thereby causing hypothermia. Conversely, higher doses of MDMA...
could conceivably result in the activation of enough 5-HT_{2A} or other nonserotonin receptors to cause hyperthermia. We found evidence supporting this hypothesis, as a 7.5 mg/kg dose of MDMA caused pure hypothermia and a 15 mg/kg dose of MDMA caused predominantly hyperthermia. On closer inspection of Fig. 1, the initial increase in temperature after 15 mg/kg MDMA is not evident until 30 min after injection, suggesting that 5-HT_{1A} receptor activation may have delayed the development of hyperthermia. We do not think this delay is secondary to drug absorption as decreases in temperature after 7.5 mg/kg MDMA are evident within 15 min of injection. These results are similar to those of other studies in which doses of 40, 20, 15, and 10 mg/kg MDMA produced either an initial hypothermia followed by hyperthermia or a delay in the development of hyperthermia (Gordon et al., 1991; Malberg et al., 1996). As doses of MDMA increase, the decrease in the initial hypothermia was likewise less (Malberg and Seiden, 1998). Data reported by Abdel-Fattah et al. (1997) support the idea that lower concentrations of serotonin may preferentially activate 5-HT_{1A} receptors, causing hypothermia. In their study they demonstrated that when rats were systemically administered the serotonin precursor tryptophan and the monoamine oxidase inhibitor pargyline developed biphasic temperature responses and that hypothalamic levels of 5HT were lower during the hypothermic phase and higher during the hyperthermic phase. Furthermore these authors showed that administering the 5-HT_{1A} antagonist pindolol prevented the hypothermic phase caused by tryptophan and pargyline (Abdel-Fattah et al., 1995). In a similar fashion, the systemic administration of WAY 100635 prevented hyperthermia evoked by the serotonin-releasing agent dexfenfluramine (Cryan et al., 2000). As hyperthermia correlates with neurotoxicity induced by MDMA, the finding in our study that 5-HT_{1A} antagonists increase hyperthermia and the corollary that 5-HT_{1A} agonists might prevent hyperthermia could lead to better selection of drugs to treat life-
threatening hyperthermia. An example is that the β-blocker propranolol has, on occasion, been suggested as a treatment for toxicity from MDMA or in cases of serotonin syndrome (Jones and Story, 2005). As propranolol is a 5-HT1A antagonist, its usage could actually result in worsening hyperthermia.

The fact that other authors have not consistently reported MDMA-induced hypothermia may be due in part to the dose of MDMA used and the method of temperature measurement. In most of the studies to date reporting MDMA-induced hypothermia at room temperature radiotelemetry was used to measure body temperature (Berkey et al., 1990; Adell and Docherty, 2006). As handling and rectal temperature measurements significantly increase body temperature and serotonin release (Berkey et al., 1990; Adell et al., 1997), animals given MDMA in studies in which these methods of temperature measurement were used may have increased amounts of serotonin release over animals in which biotelemetry was used to measure temperature. As we have previously discussed, higher concentrations of serotonin may result in the activation of 5-HT2A or nonserotonergic receptors and hence less hypothermia. Although we saw very reproducible reductions in body temperature after 7.5 mg/kg MDMA in both the dose-response experiment and the experiment in which WAY 100635 was systemically administered, the degree of hypothermia seen when microinjections preceded systemic MDMA administration (compare Figs. 1 and 2A with Fig. 5A) was less. Possible explanations for this result may be that microinjection of PBS into the rRP causes local stimulation of neurons resulting in elevations in temperature and heart rate (see Figs. 4 and 5) or the stress of the procedure itself. As the procedure requires the placement of a needle injector into a guide cannula followed several minutes later by its removal, it requires the investigator to be in proximity of the animal for a longer period of time than for a simple i.p. injection. Lending support to either of these ideas is the fact that control animals (PBS/saline in Fig 5A) microinjected with PBS in the rRP followed by i.p. saline injection had larger increases from baseline in core temperatures at 30 min (+0.8 ± 0.2°C) compared with the control animals receiving two i.p. doses of saline (saline/saline in Fig. 2) and no microinjection (+0.2 ± 0.2°C). Another possible explanation for the reduced MDMA-mediated hypothermia when drugs were microinjected is that the placement of the microinjection cannula may have damaged neurons in the rRP, reducing the hypothermia induced by MDMA. We could account for this possibility in the experiments with DPAT by making sure that animals with microinjection cannula in the rRP were capable of developing hypothermia to DPAT and eliminating those that did not from the final analysis. We did not do a similar screening procedure with MDMA as we were concerned that the neurotoxicity of MDMA might potentially affect the responses to further doses of MDMA.

Similar to other researchers, we showed that blocking 5-HT1A receptors significantly reduced MDMA-mediated locomotion (Callaway et al., 1992). That decreases in MDMA-mediated locomotion in animals pretreated with WAY 100635 occurred at 30 but not at 60 min is consistent with findings reported by Kehne et al. (1996). In their study, they suggested that early (0–30 min) locomotion induced by MDMA may be dependent on activation of 5-HT1A and dopamine D2 receptors, whereas later increases (30–60 min) depend on activation of 5-HT2A receptors (Kehne et al., 1996). Our results conflict, however, with those of McCready et al. (1999), who reported that WAY 100635 had no effect on MDMA (3 mg/kg s.c.)-mediated locomotion. Differences in our studies may be explained by the lower dose of MDMA used or the method of analysis; McCready et al. (1999) analyzed summed data over a 90-min period.

Although administration of MDMA increases heart rate and systolic blood pressure in human volunteers (Lester et al., 2000), few researchers have studied the cardiovascular effects of MDMA in animals. After administering 20 mg/kg MDMA s.c. to five Long-Evan-rats, Gordon et al. (1991) reported a steady rise in the heart rate of three animals and dramatic increases in both heart rate and temperature in two rats that subsequently died. Irvine et al. (2001) gave MDMA in a range of doses from 5 to 20 mg/kg s.c. to female Sprague-Dawley rats at a temperature of 20°C and did not report increases in heart rate and blood pressure. At a temperature of 30°C, however, all doses of MDMA tested increased heart rate and blood pressure. At a temperature of 30°C, however, all doses of MDMA tested increased heart rate and mean arterial pressure. In our study, i.p. injections of saline followed by MDMA (7.5 mg/kg) at an ambient temperature of 24 to 25°C increased heart rate but did not mean arterial pressure. Differences between the strains of rats used in our study (Sprague-Dawley) and that of Bexis and Docherty (Wistar) may explain these contrary findings (Gaudreault et al., 2001; van den Buuse and Wegener, 2005). In our study when WAY 100635 was administered, a subsequent dose of MDMA evoked a greater initial increase in heart rate and mean arterial pressure than a dose of MDMA alone. This result suggests that activation of 5-HT1A receptors initially suppresses the tachycardia and hypertension mediated by MDMA.

The results of our study reveal for the first time that activation of 5-HT1A receptors in the region of the rRP mediates, at least in part, the hypothermic response from the systemic administration of the 5HT1A receptor agonist DPAT. DPAT lowers body temperature in rats (Gudelsky et al., 1986) by inhibiting IBAT thermogenesis and decreasing tail blood flow (Ootsuka and Blessing, 2006), effects that are blocked by pretreatment with WAY 100635 (Ootsuka and Blessing, 2006b). The rRP is the principal location of sympathetic premotor neurons regulating activity of tail and pinna cutaneous vasoconstrictor nerves in rats and rabbits, respectively, and sympathetic innervation for IBAT in rats (Morrison et al., 1999; Morrison, 2004). Considerable evidence suggests that glutamate is the major excitatory neurotransmitter released by terminals of these sympathetic premotor neurons in the rRP onto thermoregulatory sympathetic preganglionic neurons in the spinal cord (Nakamura et al., 2005; Stornetta et al., 2005). However, serotonergic neurons in the rRP also project to the same sympathetic regions of the spinal cord, and markers for both glutamate and serotonin are found in some regions (Helke et al., 1997). Likewise microinjections of serotonin potentiate increases in brown adipose sympathetic nerve activity elicited by subsequent spinal microinjection of N-methyl-D-aspartate (Madden and Morrison, 2006). Serotonergic neurons in the rRP are transsynaptically labeled after injection of pseudorabies virus into the rat tail (Tóth et al., 2006) and are responsible, at least in
part, for cutaneous vasoconstrictor responses in the pinna of the rabbit that are evoked from the rRP (Otsuka and Blessing, 2006). Spinally projecting serotonergic and nonserotonergic neurons in the rRP possess 5-HT1A receptors (Helke et al., 1997). Microinjection of DPAT into the region of the rRP blocks leptin-induced thermogenesis in rats (Morrison, 2004) and cold-induced cutaneous vasoconstriction in rabbits (Otsuka and Blessing, 2006). The location of neurons containing 5-HT1A receptors involved in the cardiovascular responses to leptin and chemical disinhibition of the dorsomedial hypothalamus has likewise been reported to be in the region of the rRP and medullary brainstem (Morrison, 2004; Horuchi et al., 2005).

The results of our study demonstrate for the first time that activation of 5-HT1A receptors in the region of the rRP after a systemic dose of MDMA suppresses tachycardia but not hyperthermia. Blessing and colleagues likewise reported that inhibition of the rRP with 1 nmol of the GABA agonist muscimol did not prevent subsequent increases in sympathetic nerve activity in ear pinna of rabbits given MDMA (6 mg/kg i.v.) (Otsuka et al., 2004). The systemic administration of the 5-HT2A antagonist SR 46394B (0.1 mg/kg i.v.), however, completely reversed both the sympathetic nerve activity and vasoconstriction of the rabbit ear pinna mediated by MDMA (Otsuka et al., 2004). These data suggest that vasoconstriction mediated by MDMA occurs downstream of the rRP, probably by neurons expressing 5-HT2A receptors in the spinal cord. Whether 5-HT1A receptors involved in MDMA-mediated hyperthermia are likewise in the spinal cord is unknown. In their article, Madden and Morrison (2006) demonstrated that microinjection of 5HT into the intermediolateral column of the spinal cord, a region containing preganglionic sympathetic neurons, increased sympathetic nerve activity to IBAT after a delay of approximately 20 min. The authors presented several plausible explanations for this delay, but one area not explored was whether activation of 5-HT1A receptors may have initially suppressed sympathetic nerve activity to IBAT with later activation occurring after 5-HT1A receptor desensitization (Arana and Andrade, 1991).

In conclusion, we demonstrate that MDMA can cause hyperthermia as well as hyperthermia and that unlike DPAT, hyperthermia mediated by MDMA does not involve 5-HT1A receptors in the rostral raphe pallidus. We also demonstrate that activation of 5-HT1A receptors in the rRP suppress tachycardia mediated by MDMA. These results suggest that the thermogenic and cardiovascular effects caused by MDMA involve activation of both 5-HT1A and 5-HT2A receptors with their interactions having potential consequences for the development of hyperthermia and toxicity in humans.

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