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Effects of 3,4-Methylenedioxymethamphetamine on Autonomic Thermoregulatory Responses of the Rat[1,2]

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GORDON, C J , W P WATKINSON, J P O'CALLAGHAN AND D B MILLER *Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat* PHARMACOL BIOCHEM BEHAV **38**(2) 339–344, 1991 —3,4-Methylenedioxymethamphetamine (MDMA), a substituted amphetamine analogue which stimulates serotonin release in the CNS, has been shown to induce near lethal elevations in core temperature in the rat To characterize the effects of MDMA on temperature regulation, we measured metabolic rate (MR), evaporative water loss (EWL), motor activity (MA), and colonic temperature (T_c) in male, Long-Evans rats at 60 min following 30 mg/kg (SC) MDMA or saline at ambient temperatures (T_a) of 10, 20, and 30°C MDMA caused an elevation in MR at T_a's of 20 and 30°C but had no effect at 10°C At a T_a of 30°C, MR of the MDMA group was double that of the saline group EWL was elevated by MDMA, an effect which was potentiated with increasing T_a MDMA also elicited an increase in MA at all three T_a's MDMA led to a 3 2°C increase in T_c at 30°C, no change in T_c at 20°C, and a 2 0°C decrease in T_c at 10°C A second study found that treatment with 20 mg/kg MDMA failed to elicit an increase in blood flow to the tail in spite of a hyperthermic core temperature of 41 4°C Preliminary studies using radiotelemetry methodology suggested that MDMA lethality is preceded by precipitous elevations in heart rate and core temperature The data suggest that, at relatively warm T_a's, MDMA-induced stimulation of serotonergic pathways causes an elevation in MR and peripheral vasoconstriction, thus producing life-threatening elevations in T_c The increase in EWL following MDMA partially attenuates the hyperthermia at warm T_a's, but leads to hypothermia in the rat maintained at a cold T_a of 10°C

Metabolic rate Evaporative water loss Salivation Piloerection Hyperthermia Hypothermia
Motor activity Stimulant Ambient temperature Skin temperature Lethality Heart rate Telemetry

THE psychotomimetic properties of the amphetamine analog, 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"), have recently been assessed in several laboratories (5,20). MDMA has been found to promote [^{3}H] serotonin release from rat brain slices (12) and elicits what is known as a general serotonergic syndrome associated with a low body posture, head weaving, forepaw treading, piloerection and salivation (20).

MDMA-treated rats have been reported to have elevated body temperatures (1,15) and this response also appears to be mediated via the activation of serotonergic pathways (15). In preliminary studies we noted that MDMA-treated rats may succumb to hyperthermia, often reaching internal body temperatures in excess of 42°C immediately prior to death (Miller, unpublished observations). Considering the major role of serotonergic pathways in the regulation of body temperature in the rat and other species (10,14), it is not surprising that MDMA-treated animals undergo drastic changes in body temperature. However, there is little data on the effects of MDMA on the activity of such basic thermoregulatory parameters as metabolic heat production and heat dissipating motor outputs. The purpose of this study was to assess the effect of acute MDMA treatment on several thermoregulatory parameters in the laboratory rat, including body temperature, metabolism, evaporative heat loss, skin temperature, motor activity, and heart rate

METHOD

Animals used in this study were male rats of the Long-Evans strain obtained from Charles River Laboratories (Raleigh, NC). The animals were maintained at an ambient temperature (T_a) of 22°C, relative humidity of 50%, with a 12·12 light·dark cycle. The age of the rats was approximately 12 weeks when studied.

In the first study the effects of acute administration of MDMA

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on several thermoregulatory parameters in the unrestrained rat were determined. An environmental chamber, described in previous publications (6,8), was used to measure metabolic rate (MR), evaporative water loss (EWL), motor activity, and colonic temperature (T_c) in rats maintained at T_a's of 10, 20, or 30°C. Motor activity was measured using a 10 GHz Doppler system which essentially responds to any movement by the rat inside the environmental chamber (8). An integrating circuit summated total motor activity during the 60-min period in the chamber and was expressed in dimensions of relative units.

MDMA (HCl salt; generously supplied by NIDA) was dissolved in 0 9% saline and injected subcutaneously at a dose of 30 mg/kg in a volume of 0.1 ml/100 g body weight. Immediately following injection, the rat was placed inside the environmental chamber for 60 min, while MR, EWL, and motor activity were continuously monitored. At the end of 60 min the rat's T_c, a measure of internal body temperature, was determined by inserting a thermocouple probe 5–6 cm past the anal sphincter After removal from the chamber the rats were observed for signs of morbidity for a 24-h period. Six rats were treated with either saline or 30 mg/kg MDMA at each T_a. Body mass of the rats in this study was 468 ± 6 (S E) g.

Motor activity at the end of 60 min in the temperature gradient, T_c, and the MR and EWL data taken during the last 10 min in the environmental chamber were averaged and analyzed for statistical significance using a factorial design (18). Initially, ANOVA tests were used to test for significant dose effects at each T_a. This was followed with *t*-tests to determine the significance between the MDMA and saline treatments for each parameter.

A second experiment was designed to assess the effect of MDMA on tail skin blood flow. This was estimated by measuring tail skin temperature (T_t) and T_c in animals 3 hours after being injected with saline or MDMA and maintained in their home cages at a T_a of 21–24°C T_t was measured by attaching a thermocouple around the tail at the same time as the determination of T_c The thermocouple was secured to the tail with latex tubing and was positioned approximately half way between the tip and base of the tail This procedure lasted approximately 15 s and the animals were unrestrained throughout the procedure. Significant differences in T_t and T_c were determined using a Student's *t*-test. Body mass of the rats in this experiment was 471 ± 36 g.

In a third study, the effects of MDMA on the electrocardiogram (ECG), heart rate (HR), and body core temperature (T_{core}) were assessed using a radiotelemetry system. Note that T_{core} is used to denote the measure of internal body temperature by telemetry and is not the same measure as colonic temperature (i.e., T_c) described above. Long-Evans rats [68–74 days, 310 ± 13 (S.E) g, N = 5] were anesthetized with sodium pentobarbital (50 mg/kg; IP) and implanted with a radiotelemetry transmitter (Model TR4; Konigsberg Instruments Inc.). The transmitters were implanted in the abdominal cavity and the ECG lead wires were secured subcutaneously on the ventral thorax in order to obtain a modified lead II signal The animals were permitted access to food and water ad lib and were allowed to recover overnight.

On the day following surgery, each rat was placed in a standard Plexiglas animal cage (20 × 20 × 22 cm) with steel grid top and wood shaving bedding and with unrestrained access to food and water. The cage was placed inside a stainless steel chamber which was maintained at a T_a of 25°C. Each rat was allowed at least 60 min to acclimate to the experimental conditions before beginning the experimental protocol. After the acclimation period, baseline temperature and ECG data were collected for 30 min The rat was then injected with 20 mg/kg MDMA (SC) and the ECG and temperature data were monitored for the next 24 h

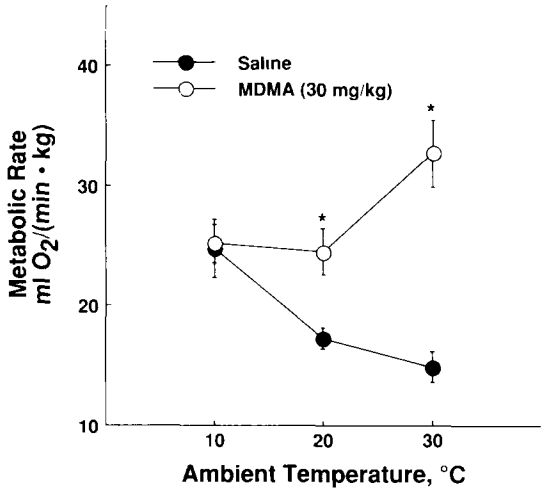

FIG 1 Effect of 30 mg/kg (SC) MDMA or saline administration on metabolic rate of the rat maintained at an ambient temperature (T_a) of 10, 20, or 30°C N = 6 per treatment at each T_a *Indicates significant difference between MDMA and saline groups

ECG and T_{core} data were obtained at 5-min intervals throughout the experimental procedure. HR was derived from the ECG. Data acquisition was fully automated and controlled by a dedicated minicomputer. Each animal was used as its own control and HR values for each rat were normalized with respect to baseline values averaged over the 30-min control period Rats were unrestrained throughout the experimental procedure and allowed to roam freely within their cages.

RESULTS

MDMA exerted profound effects on several thermoregulatory parameters causing them to be highly susceptible to changes in T_a (Fig. 1). MR was significantly increased at T_a's of 20 ($p<0.006$) and 30°C ($p<0.0002$) relative to the controls but was unaffected at 10°C The increase in MR of the MDMA-treated rats was 41 and 118% over that of the saline group at T_a's of 20 and 30°C, respectively. EWL was largely unaffected by T_a in the saline-treated rats, whereas the MDMA-treated rats exhibited a near linear increase in EWL with increasing T_a (Fig. 2) The elevation in EWL following MDMA was significantly greater than that of the saline group at 10 ($p<0$ 002), 20 ($p<0.001$) and 30°C ($p<0.0001$). The effect of MDMA on EWL was more striking than its effect on MR; for example, MDMA administration led to a 180% elevation in EWL over that of the saline group at a T_a of 30°C.

Motor activity in the saline group increased linearly with decreasing T_a (Fig. 3). MDMA elicited significant elevations in motor activity at T_a's of 10 ($p<0.0008$), 20 ($p<0$ 0001), and 30°C ($p<0.0001$). The absolute level of motor activity in the MDMA group was similar at each T_a, however, because the rat normally increases its motor activity as T_a decreases below the thermoneutral zone (7), the relative increase in activity after MDMA treatment was reduced with decreasing T_a. T_c was unaffected by MDMA at a T_a of 20°C ($p<0.93$), significantly reduced by 2.0°C when exposed to a T_a of 10°C ($p<0.0009$), and significantly elevated by 3.2°C when exposed to a T_a of 30°C ($p<0.0001$) (Fig 4) All animals treated with MDMA displayed stereotypies of lateral head movement. In addition, piloerection of the fur was noted in all MDMA-treated rats.

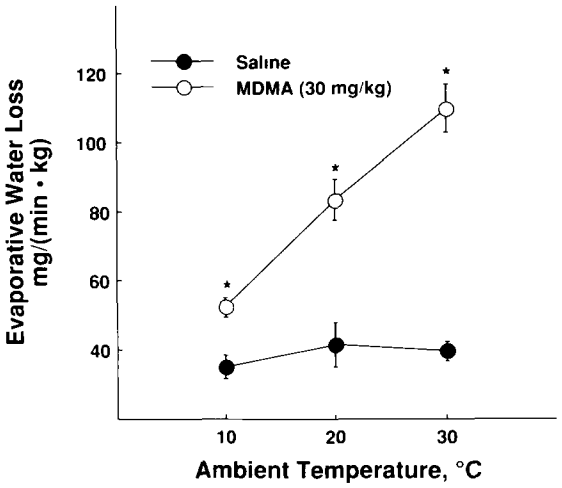

FIG 2 Effect of 30 mg/kg (SC) MDMA or saline on evaporative water loss (EWL) as a function of T_a These data collected from the same animals as that of Fig 1 Abbreviations same as Fig 1

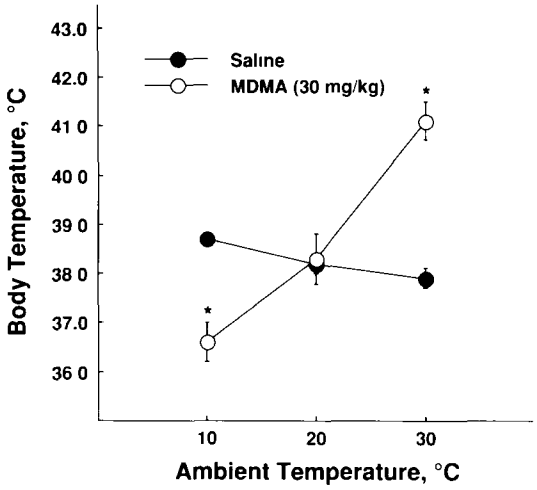

FIG 4 Effect of 30 mg/kg (SC) MDMA or saline on colonic temperature (T_c) as a function of T_a These data collected from the same animals as that of Fig 1 Abbreviations same as Fig 1

The tremendous increase in metabolism at 20 and 30°C along with hyperthermia at 30°C following MDMA administration noted here and elsewhere (15) prompted the experiment to estimate tail blood flow following MDMA treatment (Fig. 5). Three hours after injection, T_t and T_c of surviving animals administered 20 mg/kg MDMA was 23.3 ± 0.1 (S.E.) °C and 41.5 ± 0 6°C, respectively On the other hand, rats treated with saline had a relatively normal T_t of 25.5 ± 0.4°C and a T_c of 37.5 ± 0.1°C Some of the MDMA-treated rats died before any temperature data could be collected. These were included into the overall mortality data (see below) but were not included in the collection of temperature data. For comparison of the MDMA response to a positive control, an increase in tail blood flow was achieved by placing 7 rats in the environmental chamber maintained at a T_a of 37°C for 60 min. This procedure resulted in a T_c of 40.3 ± 0.2°C and a T_t of 31.3 ± 0 3°C (Fig. 5).

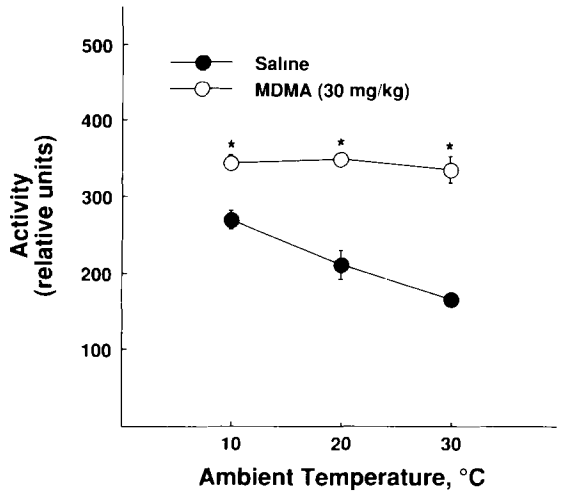

FIG 3 Effect of 30 mg/kg (SC) MDMA or saline on motor activity as a function of T_a These data collected from the same animals as that of Fig 1 Abbreviations same as Fig 1

In the studies utilizing the environmental chamber, percent mortality at 24 h postinjection of MDMA was 33, 33, and 100% for animals maintained at T_a's of 10, 20, and 30°C, respectively (Fig. 6) In the skin temperature studies, percent mortality with a 20 mg/kg dose of MDMA was 66%, with the deaths occurring within approximately three hours postinjection

Preliminary results using the radiotelemetry procedure demonstrated that MDMA causes rapid elevation in heart rate (HR) and T_{core}, effects which may be related to MDMA-induced lethality. Prior to injection of MDMA the baseline HR and T_{core} was 365 ± 14 bpm and 37.8 ± 0.3°C, respectively. Administration of 20 mg/kg MDMA led to abrupt elevations in HR and T_{core}. Three of the five rats treated with MDMA died within 5.5 h following injection In all three cases, HR rose to over 600 bpm and T_{core} exceeded 42°C (Fig. 7). ECG waveforms demonstrated occasional missed or delayed beats, amplitude changes, and general muscle artifacts consistent with the constant stereotypies and hyperactivity as described in the above experiments. In contrast, the two rats which survived the MDMA treatment reached maximum HR's of only ~470 bpm and maximum T_{core} did not exceed 41.0°C These two animals exhibited a more restrained locomotion in the cage but displayed the typical MDMA-induced stereotypies T_{core} of the two survivors did not return to baseline values (i e., until 9 h postinjection), HR values, while more variable, reached control values within 8 to 11 h postinjection Although not shown, we have found that control animals given saline undergo transient elevations in %HR and T_{core} lasting approximately 30 min with full recovery within 60 min postinjection

DISCUSSION

Overall, MDMA exerted profound effects on the thermoregulatory system of the rat with many of the effects being highly dependent on T_a MDMA elicited a massive increase in metabolism in the rat maintained at a T_a of 30°C which is undoubtedly responsible for the extreme hyperthermia after only 60 min postinjection. In comparison, rats acclimated to thermoneutral conditions and administered a sympathomimetic such as norepinephrine sustain, at the most, a 25% elevation in MR above basal levels (11). The piloerection of the fur noted here and elsewhere (20)

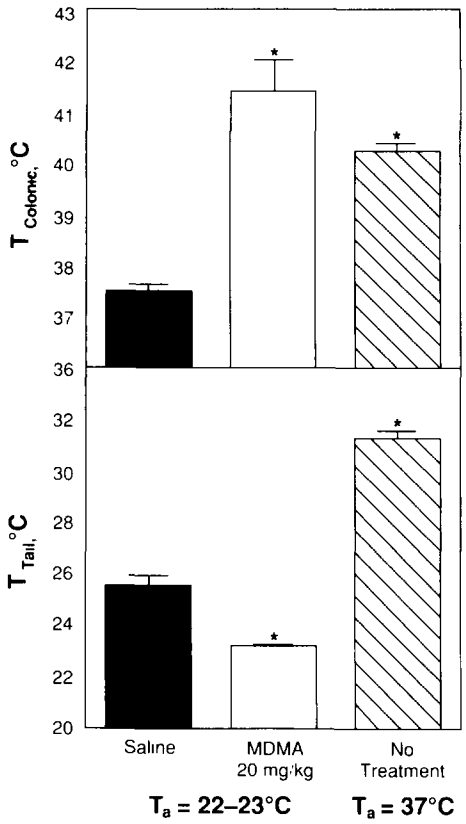

FIG 5. Effect of 20 mg/kg (SC) MDMA or saline on T_c and tail skin temperature (T_c) in rats maintained in their home cages for 3 hours postinjection For comparison, T_c and T_t of rats maintained in environmental chamber at T_a of 37°C for 60 min are also presented. N = 6 for MDMA group, N = 7 for saline group, and N = 7 for warm T_a group

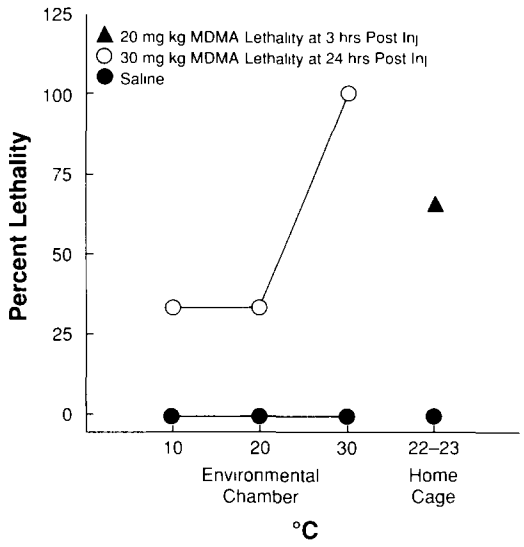

FIG 6. Effect of T_a on lethality following MDMA administration of 20 or 30 mg/kg (SC)

following MDMA treatment further exacerbates the hyperthermic condition by attenuating heat dissipation. A most important observation is that the MDMA-treated rat in a hyperthermic state fails to increase blood flow to the tail, a common thermoregulatory response in the rat when body temperature is elevated via exercise (19), ambient heat stress (17), or through pharmacological manipulation (13). Interestingly, tail skin temperature of amphetamine-treated rats is also reported to remain below that of saline-treated animals at T_a's of 15 to 37°C (23).

The increase in EWL following MDMA facilitates heat dissipation and most likely alleviated some but not all of the increase in body temperature observed at a T_a of 30°C. This effect on EWL may be attributable to the fact that MDMA stimulates salivation in the rat (20). Since the hyperthermic rat increases EWL via grooming of saliva on the fur [for review, see (7)], it could be argued that the MDMA-induced salivation is an indirect result of the compound's effect on body temperature. However, the sustained elevation in EWL in rats maintained at a T_a of 10°C in the face of a significant reduction in T_c provides strong evidence for a direct effect of MDMA on saliva secretion in the rat. Furthermore, the increase in EWL may also account for the significant decrease in body temperature following MDMA treatment at a T_a of 10°C. At this T_a MDMA had no effect on MR; thus, assuming that MDMA did not increase sensible heat loss through peripheral vasodilation, it can be concluded that the significant increase in EWL was a major factor leading to hypothermia.

The pharmacological properties of MDMA are generally consistent with models for the neurochemical control of body temperature in the rat, however, there are some discrepancies. The serotonergic pathways are crucial in temperature regulation. Generally, local administration of 5-HT into the anterior hypothalamic area results in heat production/conserving responses which lead to an elevation in body temperature (10,14). When administered acutely, MDMA causes massive release of 5-HT and subsequent stimulation of serotonergic pathways in the CNS, effects which may also be responsible for the major behavioral effects of MDMA (15). Although the neurochemical mechanisms of thermoregulation are complex, involving multiple neurotransmitters and other modulating substances, the above data suggest that MDMA-induced release of 5-HT may account for the generalized response of the rat to elevate body temperature. On the other hand, there is substantial evidence for MDMA-induced release of dopamine in the CNS (9,21). Moreover, microinjection of dopamine in the thermoregulatory centers of the CNS generally elicits a reduction in body temperature (2,3), although there are a few exceptions where dopamine microinjection led to an elevation in temperature [for review, see (2)]. The MDMA-induced increase in salivation noted here and elsewhere (20) is a heat dissipating thermoregulatory response and also does not fit in with the general serotonergic mechanisms of temperature regulation in the rat. Hence, the neuropharmacological properties of MDMA explain many but not all of MDMA's effect of thermoregulation in the rat.

The thermoregulatory effects of MDMA are similar in some respects to that elicited by amphetamine. Rats administered amphetamine generally undergo substantial elevations in metabolic rate and body temperature; however, the effects are often variable and are also highly dependent upon T_a (22,23). Generally, cold T_a's (ca. 4 to 15°C) lead to hypothermia, whereas an extremely warm T_a of 37°C leads to severe hyperthermia in the amphetamine-treated rat. Obviously, the prevailing T_a can influence the direction of change in body temperature following exposure to MDMA as well as amphetamine (23). An observation which may be pertinent to this discussion is the recent study of Preston et al. (16) who found that acute administration of the amphetamine cogener, fenfluramine, stimulated thermogenesis at a relatively warm T_a of 28°C, but inhibited thermogenesis in the cold (T_a = 4°C).

The 100% mortality of rats given MDMA at a T_a of 30°C

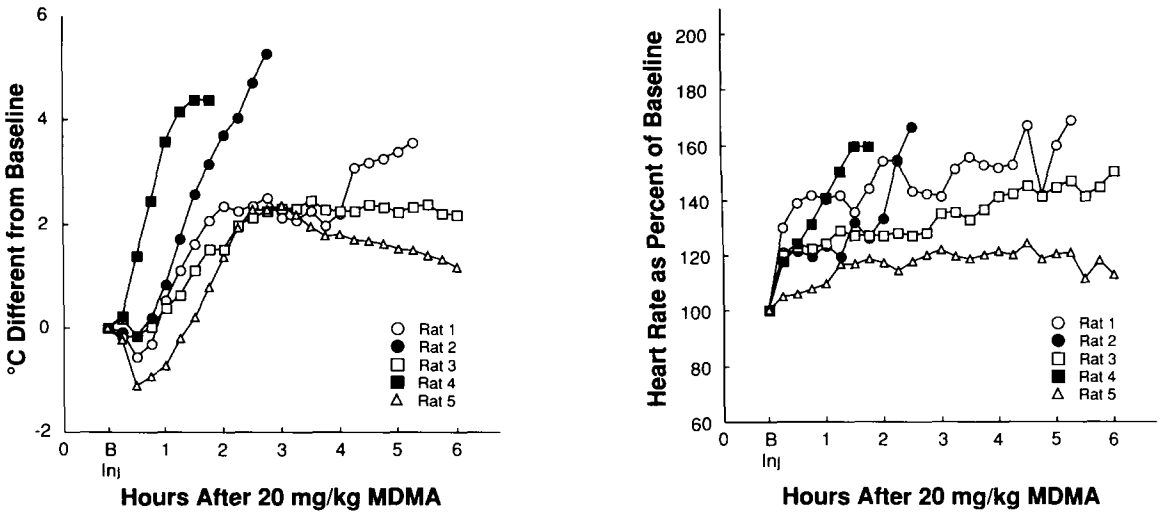

FIG 7 Time-course of change in T_{core} and % change in HR of five rats injected with 20 mg/kg (SC) and maintained at a T_a of 25°C Rats were permitted to acclimate to the experimental conditions for 90 min prior to MDMA administration Rats 1, 2, and 4 died following collection of the last plotted data points

suggests that the thermoregulatory system is involved in the manifestation of MDMA toxicity We have found that animals on the verge of MDMA-induced death have T_c's of 42°C which is 1 to 2°C below the lethal core temperature of animals subjected to acute heat stress (4,7).

It is also important to consider why the thermoregulatory response to MDMA differed considerably between rats maintained in the environmental chamber at a T_a of 20°C and rats placed in their home cages maintained at a similar T_a (~22°C). Most likely, rats in the environmental chamber, which is a water-perfused metal jacket, dissipate heat much more efficiently than rats placed in a plastic-constructed home cage lined with wood shavings. Thus the efficacy of MDMA to elevate body temperature is greatest under conditions where heat loss to the environment is impeded, which in the case of this study was achieved by either elevating T_a or adding insulation (i.e., wood shavings) to the cage. The ambient thermal environment also had a major impact on the extent of MDMA-induced lethality suggesting a relationship between MDMA-induced hyperthermia and it lethal effects. These data may prove to be a problem in the replication of MDMA effects in other laboratories

The radiotelemetry data, which, because of the small sample size, are presented here as preliminary results, are the first data on the effects of MDMA in the unstressed and unrestrained animal. These data suggest that MDMA-induced lethality is preceded by a tremendous increase in core temperature and that the increase in HR seems to follow the temperature responses. At this point the cause of MDMA lethality is unknown, however, the combination of massive increases in T_{core} and HR may manifest as lethality.

Overall, acute administration of MDMA in the rat maintained at a relatively warm T_a activates an array of thermoregulatory effectors including increased metabolic heat production, peripheral vasoconstriction, and piloerection with the consequences of a precipitous elevation in deep body temperature. The elevation in EWL following MDMA treatment facilitates heat loss However, when maintained in typical home cages at standard room T_a's the effects of MDMA on heat producing/conserving responses clearly outweigh the effects on heat dissipation, the net result being profound hyperthermia This response may be a useful model for studying the role of the serotonergic pathways in the control of body temperature. Furthermore, the striking effect of MDMA on autonomic thermoregulatory processes will undoubtedly affect the manifestation of commonly reported MDMA-induced behavioral effects, especially in the face of changes in T_a.

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