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ORIGINAL INVESTIGATION

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Persistent loss of thermoregulation in the rat induced by 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") but not by fenfluramine

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Abstract Using radio-biotelemetry, the timecourse of recovery and sensitivity to ambient temperature (Ta) of the thermogenic response of methylenedioxymethamphetamine (MDMA or "Ecstasy") was examined. Ambient temperatures of 17 and 22°C produced very different response profiles, with the lower temperature producing a hypothermic response to 10 and 15 mg/kg doses of MDMA, and the higher temperature producing a profound hyperthermia to the same doses. Although the peak responses to the drug had subsided within 5 h of administration, residual effects, in the form of an elevation of body temperature during the "low" phase of the diurnal cycle, were present for a further 48 h. Long-lasting disruption of the thermoregulatory system following a short series of MDMA administrations (10 mg/kg once per day for 4 days) was shown by exposing the rats in the undrugged state to a thermoregulatory challenge, consisting of 60-min exposure to a Ta of 30°C, at 1 week before, and at 4 weeks and 14 weeks after the drug administration. MDMA-treated rats showed a prolonged hyperthermic response to the challenge at both post-drug intervals compared with fenfluramine-treated rats and saline-treated controls. Thus, the results indicate both that MDMA's thermogenic effects are more sensitive to Ta than previously demonstrated, and that the serotonergic neurotoxicity of the drug may produce long-lasting changes in thermoregulatory mechanisms.

Key words MDMA · Fenfluramine · Thermoregulation · Ambient temperature

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Introduction

Amphetamine, and its substituted forms methamphetamine, methylenedioxymethamphetamine (MDMA), and fenfluramine, can produce substantial depletions of the neurotransmitters serotonin (5-HT) and dopamine (DA), and/or their uptake sites, throughout the brain of animals (Ricaurte et al. 1985; Commins et al. 1987; Battaglia et al. 1988; Hewitt and Green 1994). In some cases, these changes have been related to neurodegeneration of the 5-HT and DA neurons using visualisation, immunocytochemical and reactive gliosis techniques (Molliver et al. 1990; Miller and O'Callaghan 1995). There is considerable concern about the degree to which these effects generalise to humans, particularly in the case of MDMA ("Ecstasy"), which is a widely used recreational drug (reviewed in Green et al. 1995) and fenfluramine, which has been extensively prescribed as an anti-obesity drug (Weintraub 1992).

The profound effect of the substituted amphetamines on CNS transmitter levels also has acute physiological consequences, including hyperthermia, which is mediated primarily through serotonergic input to the hypothalamo-pituitary-adrenocortical axis, and which in the case of MDMA, has been responsible for a number of deaths and cases of serious illness. Temperatures as high as 43°C have been recorded in hospital emergency rooms (Henry 1992; Randall 1992), and rapid treatment to reduce core temperature using drugs such as dantrolene or administration of cold IV fluids is often required (Singarajah and Lavies 1992; Watson et al. 1993). Recently, there has been considerable interest in the degree to which the acute effects of these drugs, particularly thermogenesis, contributes to the neurotoxicity. The direction and timecourse of the thermogenic response of MDMA is complex and partly determined by the prevailing ambient temperature (Ta). Generally, higher doses and a higher Ta produce hyperthermia, lower doses and a lower Ta

produce hypothermia, while intermediate levels of dose and Ta may produce biphasic response patterns of hypothermia followed by hyperthermia (Gordon et al. 1991; Dafters 1994; Malberg et al. 1996). For example, a single administration of MDMA which produces a hyperthermic response of 2.0°C at an ambient temperature of 24°C produces a hypothermic response of 1.5°C at an ambient temperature of 11°C (Dafters 1994). Treatments which reduce the hyperthermic effect of MDMA, such as lowering Ta or coadministering other drugs such as haloperidol, diazepam and dizocilpine, can protect against subsequent serotonergic and dopaminergic neurotoxicity (Bowyer et al. 1994; Farfel and Seiden 1995a,b; Miller and O'Callaghan 1995; Malberg et al. 1996). A similar Ta dependence has been reported for fenfluramine-induced thermogenesis (Preston et al. 1990).

Despite the importance of the thermogenic effects of the substituted amphetamines, both in terms of their immediate consequences and their possible involvement in neurotoxicity, very little is known about the detailed timecourse of the acute response, its sensitivity to the Ta-modulation effect, or the recovery period following drug administration. In addition, no study to date has investigated whether these drugs can produce a relatively permanent change (i.e. lasting weeks or months) in normal thermoregulatory mechanisms. The research described here addresses each of these issues. We employed a remote, radio-telemetry system to monitor continuously body temperature and activity levels in response to pharmacological and other experimental procedures. The advantages of this technique have been described previously (Dafters 1994), but include the elimination of confounding effects of hyperthermia induced by the stress of restraint and rectal measurement procedures employed in traditional techniques, the ability to monitor responses at high frequency and for extended periods on a 24 h/day basis, and the capacity to target drug administrations to a specific phase of the diurnal temperature cycle.

Materials and methods

Animals, drugs and apparatus

Female Wistar rats, obtained from B & K Supplies Ltd. Hull, UK, and weighing 200–250 g, were housed individually in plastic cages (40 × 25 × 20 cm) with free access to food and water, and maintained in holding rooms at a temperature of 22 ± 1°C and on a standard 12:12 h light:dark cycle. 3,4-Methylenedioxymethamphetamine and fenfluramine hydrochloride, purchased from Sigma Chemical Co. Poole, Dorset, UK, were dissolved in 0.9% physiological saline and injected subcutaneously in the neck. Temperature and activity were monitored remotely and continuously at 10-min intervals throughout the experiment by means of a computer-controlled biotelemetry system (Dataquest III, Mini-Mitter Co. Inc., Oregon, USA). The system has been described fully previously (Dafters and Taggart 1992) but briefly, wax-coated transmitters (Mini-Mitter model VM-FH) are implanted under anaesthesia (Hypnorm/hypnovel) in the rats abdominal cavity, emit radio-frequency pulses (range 300–600 Hz) whose frequency varies linearly with temperature in the physiological range. Receivers (model RA1010) located beneath each cage transmit the frequency information to a computer which records and analyses the data. Moment-to-moment changes in position and orientation of the transmitters can also be detected and translated into activity counts. Where ambient temperatures above the standard animal room level of 22°C were required (as in the thermoregulatory challenge), they were achieved by placing rats individually into polystyrene boxes (30 × 30 × 30 cm) equipped with a thermometer, with side holes for ventilation, and with an open metal grill as a lid through which radiant heat was applied from a single 40 W tungsten lamp. The temperature inside the boxes was maintained at the desired level (± 1°C) by regular minor adjustment to the height of lamp. The boxes were placed on top of the receivers so that body temperature could continue to be monitored telemetrically in the usual way.

Procedures

Sensitivity of MDMA thermogenesis to Ta

Previous studies showing effects of Ta on the thermogenic response of MDMA in rats have used fairly extreme ambient temperature ranges, for example, 24 versus 11°C (Dafters 1994) or 10 versus 20 versus 30°C (Gordon et al. 1991). To see whether the thermogenic response was sensitive to more subtle changes in Ta, we monitored the effects of MDMA (10 or 15 mg/kg) administered at 22 or 17°C. Rats were divided into two groups ($n = 6$), one which received a single dose of 10 mg/kg MDMA at Ta = 17°C, and, 1 week later, a single dose at Ta = 22°C. The other group received doses of 15 mg/kg at each Ta. The standard maintenance temperature of the animal colony is 22°C and therefore required no additional procedures. The Ta = 17°C condition was achieved by turning off the heating to the experimental room and temporarily opening an external door (outside temperature was approximately 8°C). When the room temperature had dropped to 17°C it was maintained (to ± 1°C) by periodic operation of the heating system. The ambient temperature was maintained for a period from 1 h before the drug administration to 3 h after the administration.

Duration of MDMA-induced hyperthermia

The timecourse of recovery of normal diurnal body temperature rhythm following the second dose of drug was monitored by recording body temperature on the day before drug administration, the drug administration day itself, and for the 3 subsequent days. To quantify recovery, the mean body temperature during the 6-h period (1400–2000 hours) was calculated for the day of administration and for each of the 3 following days, and each of these was compared with the mean of the day preceding the administration (i.e. the baseline) using the *t*-test (one-tailed) for paired samples.

Long-term changes in thermoregulation

To detect possible long-term changes in thermoregulatory processes which might result from the serotonergic neurotoxicity of the substituted amphetamines, the hyperthermic response of undrugged rats to a thermoregulatory challenge (Ta = 30°C for 60 min) was measured at 1 week before, and at 4 weeks and 14 weeks following dosing with either MDMA or fenfluramine (10 mg/kg per day for 4 consecutive days). This dose regimen was selected because it is known to exceed the minimum level required for neurotoxicity

of the two substances (McCann et al. 1994; Colado et al. 1995), and also because at this dose and ambient temperature, fenfluramine, like MDMA, is known to produce hyperthermia (Sulpizio et al. 1978; Pawlowski 1981; Sugrue 1984). To test for possible effects of Ta on the drugs' long-term effects, the repeated drug administrations were given at 22°C in some rats and at 28°C in others. The temperature was maintained for 60 min commencing at the time of injection. Twenty-four rats were divided into three groups ($n = 8$) which received either MDMA, fenfluramine or saline. Following the initial baseline thermoregulatory challenge, half of each group received their four drug administrations at 22°C and half at 28°C. Four weeks and 14 weeks following the last drug administration, all subjects received an additional thermoregulatory challenge. In order to quantify this effect the "area-under-the-hyperthermia-curve" was calculated for each group for each challenge. This measure incorporates both the magnitude and duration of the response, and was calculated for each animal in following way. At each 10-min interval, the temperature on the heat challenge session was compared with that on the previous session (i.e. the differences between each filled and unfilled column in Fig. 3). The duration over which those differences were significant ($P < 0.05$, t-test for paired samples) was then determined (marked by the vertical lines in Fig. 3). This established the temporal boundaries of the hyperthermic response for each rat. The area of hyperthermia within this region (in degree-minutes) was then calculated by summing the products of the temperature difference at each time bin and its width (10 min). Finally, two difference scores were calculated for each animal by subtracting the pre-drug "area" from each of the two post-drug "areas". These difference scores were then subjected to a $3 \times 2 \times 2$ way MANOVA, with Drug (MDMA, fenfluramine or saline), Administration temperature (28 or 22°C), and Post-drug time (4 weeks or 14 weeks) as factors.

Results

Effects of Ta

Figure 1 shows the acute effect on body temperature of 10 and 15 mg/kg MDMA administered at an ambient temperature of 22 or 17°C. In each graph, the data from the day preceding the second drug administration is included to illustrate the normal undrugged diurnal pattern of response and to provide a baseline against which to assess the level of hyperthermia or hypothermia. The effect of Ta is apparent at both dose levels. The dominant response at 22°C is hyperthermia (peak response of +2.32°C and +1.36°C in the 15 and 10 mg/kg groups, respectively). At 17°C the dominant

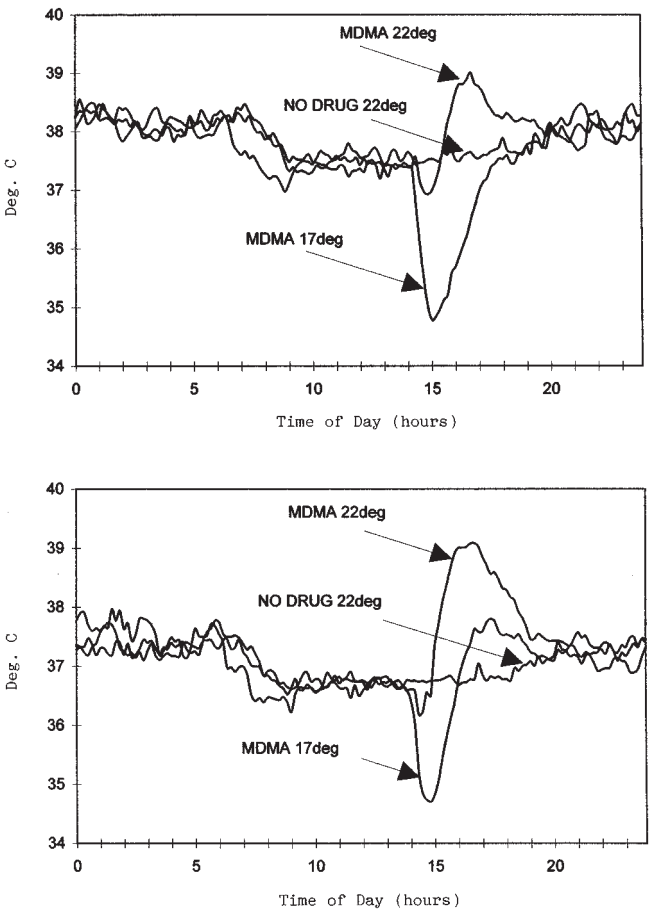

Fig. 1 Mean body temperature (°C) in rats receiving 10 mg/kg (*upper*) and 15 mg/kg (*lower*) MDMA at ambient temperatures of 22°C and 17°C. The drug was administered at 1400 hours in each case

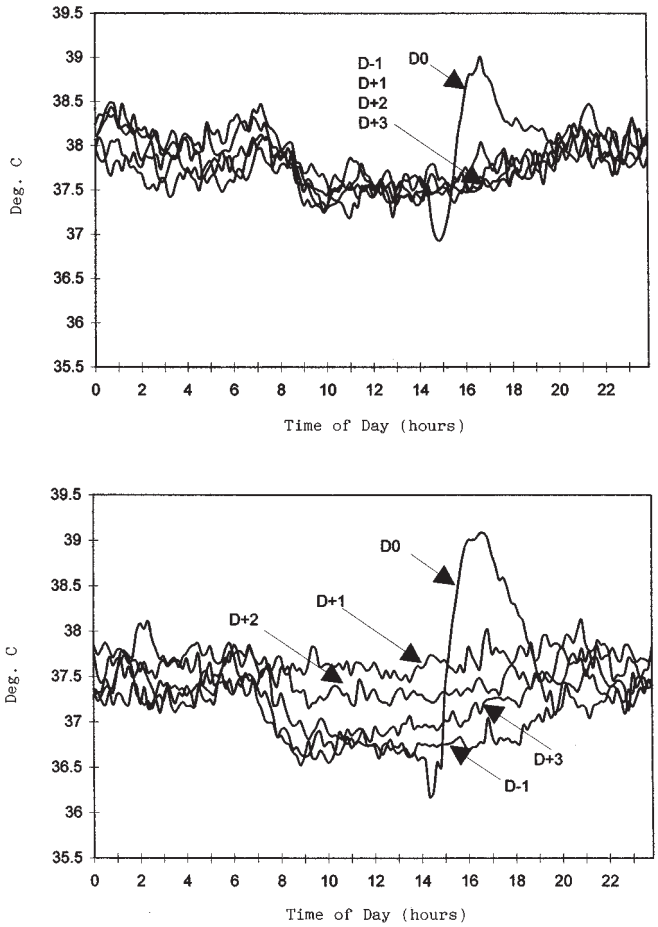

Fig. 2 Mean body temperatures (°C) in rats receiving 10 mg/kg (*upper*) and 15 mg/kg (*lower*) MDMA on the drug administration day (*D0*), the day preceding drug administration (*D-1*), and the three days following drug (*D+1*, *D+2*, *D+3*). Ambient temperature was 22°C in all cases

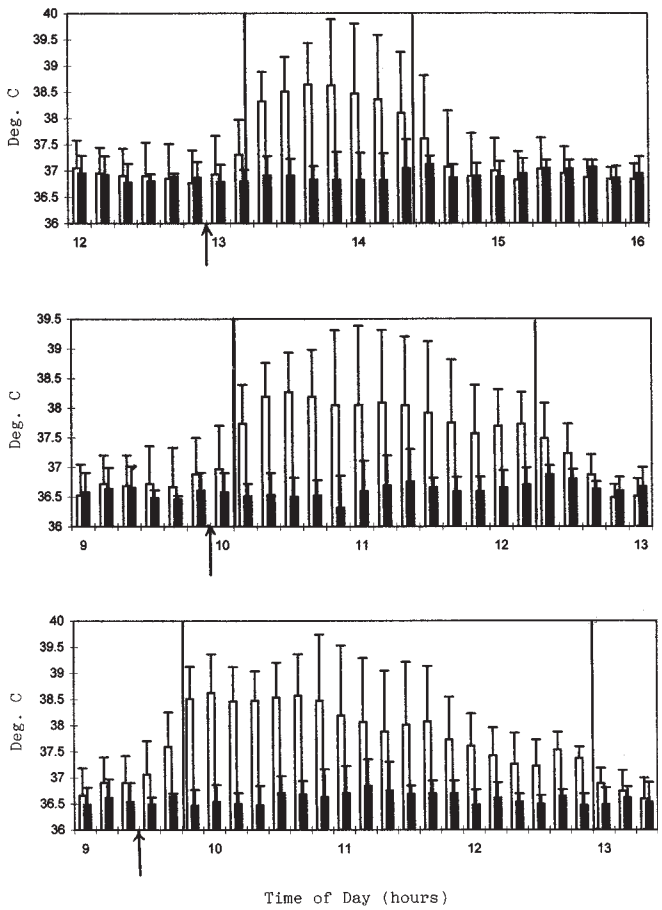

Fig. 3 Body temperature response (deg C + SD) during thermoregulatory challenge in rats treated with 10 mg/kg MDMA (four doses in 4 days). Data is plotted at 10-min intervals over a 4-h period with the start of the challenge test indicated by *arrows*. *Upper*, pre-drug test; *middle*, 4 weeks post-drug test; *lower*, 14 weeks post-drug test. The *vertical lines* enclose the region in which the body temperature on the test day (*unfilled columns*) exceeds that at the same time on the preceding day (*filled columns*) – see text for further explanation

response is hypothermia (peak response is −2.05 and −2.75°C in the 15 and 10 mg/kg groups).

Duration of thermogenesis

Figure 2 shows the pattern of recovery of diurnal temperature rhythm which follows the second drug administration (at Ta = 22°C). The lower graph shows that the 15 mg/kg dose, which produced a peak hyperthermic response of 2.32°C, has a prolonged effect on the diurnal temperature pattern. The elevation of body temperature was significant on the drug day ($P < 0.005$), on drug day + 1 ($P < 0.005$), and drug day + 2 ($P < 0.01$), and non-significant for drug day + 3. Similar comparisons in the 10 mg/kg group, where the peak hyperthermic response was only 1.36°C, revealed no significant differences between the pre-drug baseline and any subsequent day.

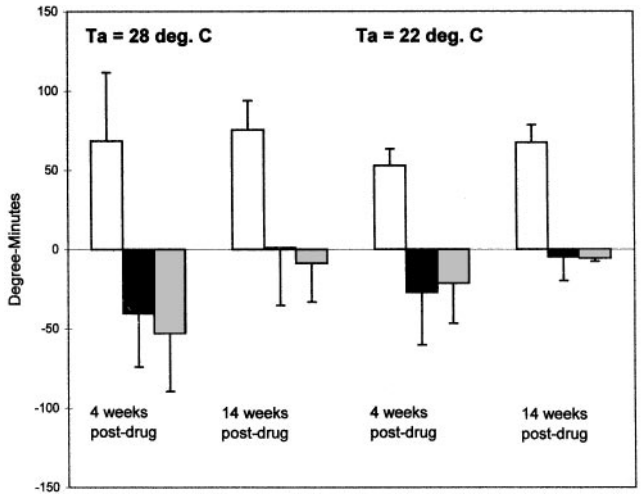

Fig. 4 Mean area-under-the-hyperthermic-curve (+SD) of undrugged animals on thermoregulatory test, expressed as postdrug – predrug difference scores, in groups given MDMA (10 mg/kg), fenfluramine (10 mg/kg) or saline at 28°C or 22°C. □ MDMA, ■ fenfluramine, ▒ saline

Long-term changes in thermoregulation

Figure 3 plots the mean temperatures for the MDMA (high administration temperature) group on the predrug and the 2 post-drug challenge days against the baseline response of the previous day. Note the prolongation of hyperthermia on the two post-drug challenge sessions. A similar pattern of results was also seen in the MDMA (low administration temperature) group but not in either of the fenfluramine or saline groups.

The MANOVA on the area-under-the-curve difference scores (plotted in Fig. 4) revealed that the main effect of Drug was highly significant [$F(2,18) = 42.99$, $P < 0.0001$], as was the main effect of Post-drug time [$F(1,18) = 12.99$, $P < 0.01$]. No other main effects or interactions were significant ($P > 0.05$, in each case). A Scheffe test showed that the increased hyperthermia in MDMA rats at both post-drug intervals was significantly greater than in the fenfluramine and saline subjects ($P < 0.005$, in each case), which did not differ from each other. The MDMA-induced increase in hyperthermia at 14 weeks post-drug was not significantly different from that at 4 weeks post-drug. The significant main effect of Post-drug time may be accounted for by the fact that in all three groups, there was a trend towards increased positivity in the difference scores between the 4-week and 14-week tests.

Discussion

The data reported here have shown that the thermogenic response of MDMA is exquisitely sensitive to the prevailing ambient temperature. A decrease in Ta

of only 5°C (from the standard colony temperature of 22°C) was sufficient to change the effect of 10 or 15 mg/kg of the drug from hyperthermia to hypothermia. To our knowledge, this is the first evidence that such a small change in Ta can reverse the direction of the thermic response of MDMA, although a similar reversal to a 5°C change in Ta has been reported for fenfluramine (Preston et al. 1990). It was also shown here that the higher (15 mg/kg) dose had a protracted hyperthermic effect which was still manifest as an abnormally elevated "low" phase of the diurnal rhythm 48 h after drug administration. The fact that this prolonged effect is only evident from a detailed study of the circadian temperature rhythm, where it is manifest as an increase in the low phase of the cycle, may be taken as a vindication of the use of biotelemetry where frequent and prolonged measurements are easily taken.

The extreme sensitivity to ambient temperature shown here strongly suggests an effect on the thermoregulatory set-point mechanism, and this is compatible with the known pharmacological properties of MDMA and other substituted amphetamines. Acute administration of these drugs produces an initial massive surge in release of serotonin (5-HT) and subsequent stimulation of serotonergic pathways in the CNS (Green and Colado 1995). Serotonergic pathways are known to play a crucial role in thermoregulation, and local administration of 5-HT into the anterior hypothalamic area results in heat production/conservation processes which lead to a rise in body temperature (Jacob and Girault 1979; Myers 1980).

A novel and important extension to the existing literature, is the finding that the effect of a short series of MDMA administrations on the thermoregulatory system, in the form of an exaggerated response to an environmental heat challenge, was still apparent fourteen weeks later. This suggests that some relatively permanent change in the responsible neural mechanisms has occurred which may be a manifestation of serotonergic neurotoxicity. If this is so, it suggests that there may be significant differences in the mechanisms and/or locus of neurotoxicity within the class of substituted amphetamines, since fenfluramine did not produce a similar lasting change in thermoregulation. There is other recent evidence that MDMA and fenfluramine may not have identical neurotoxic profiles. For example, whereas MDMA-induced serotonergic neurotoxicity may involve free radical formation, fenfluramine-induced neurotoxicity apparently does not (Murray et al. 1996; Colado et al. 1997), and in the mouse, MDMA has been shown to destroy dopaminergic projections to the striatum whereas fenfluramine does not (Miller and O'Callaghan 1994, 1995).

There are some clinical implications of these findings. Differences between the effects of MDMA and fenfluramine weaken the argument that recreational use of MDMA does not cause damage because fenfluramine has been widely and apparently safely used as an anorectic drug for many years (Saunders 1996). In addition, although it is not known to what extent the findings reported here may extend to human recreational users, it is at least possible that long-term disturbances in thermoregulation, perhaps leading to a proneness to exertional heatstroke, must be added to the list of potential hazards associated with MDMA use. On a more positive note, it is possible that changes in thermoregulation capacity assessed by some kind of thermal challenge test, could be used as an early clinical marker of compromised serotonergic function in the human brain following chronic drug use. This is a matter of priority, since reliable markers of the integrity of 5-HT neurons have so far proved elusive, although recent PET imaging studies using new selective radioligands which bind to 5-HT transporters are a promising development (Szabo et al. 1995).

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