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Review

A review of the mechanisms involved in the acute MDMA (ecstasy)-induced hyperthermic response

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Abstract

The predominant severe acute adverse effect following ingestion of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) by recreational users is hyperthermia which can induce other associated clinical problems and occasionally death. There is no pharmacologically specific treatment. MDMA also induces dose-dependent hyperthermia in experimental animals. This review examines the consequences of MDMA administration on body temperature in humans and rodents. In rats hyperthermia results primarily from dopamine release and is influenced by dose, ambient temperature and other housing conditions. The response is increased in rats with a prior MDMA-induced neurotoxic lesion of 5-hydroxytryptamine (5-HT) nerve endings. Increased MDMA-induced locomotor activity appears to play no role in the hyperthermic response. However, the size of the acute hyperthermic response plays a major role in determining the severity of the subsequent neurotoxicity. These results suggest that any MDMA-induced hyperthermic response will be enhanced in hot, crowded dance club conditions and that ingesting the drug in such conditions increases the possibility of subsequent cerebral neurotoxic effect.
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Keywords: 3,4-Methylenedioxymethamphetamine; Ecstasy; Hyperthermia; Neurotoxicity; Serotonin; Body temperature

Contents

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1. Introduction

Administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') produces two major effects in experimental animals. The acute effect is a rapid hyperthermia which can last several hours and which is associated with monoamine release from nerve endings in the brain. The second effect is a long-term neurotoxic loss of 5-hydroxytryptamine (5-HT) in the forebrain of rats, guinea pigs and primates (see Green et al., 2003 for review). Substantial research activity has been expended in trying to understand the mechanisms involved in producing the neurotoxic damage and investigating whether the 5-HT loss also has any long-term functional consequences in terms of mental health of users of MDMA, since it is a popular recreational drug with young people. If persons taking MDMA recreationally also suffer long-term neurotoxic damage to 5-HT nerve endings in the brain then they may also subsequently experience both physiological and psychiatric problems given the major neurotransmitter role played by 5-HT. At present there is no unequivocal evidence for any long-term effects of MDMA ingestion in humans, although there are now enough data collected in human studies to give rise to concerns that high or repeated doses of MDMA taken recreationally can produce long-term health problems (see Green et al., 2003). The fact that the existence, or otherwise, of long-term clinical problems in persons who have used MDMA remains controversial is related to two major factors. The first is that prospective studies are clearly unethical and therefore retrospective studies are required. Such studies are always problematic with illicit recreational drugs. Lack of knowledge of the doses taken, the frequency of use, the purity of the drug ingested and the use (concurrent or consecutive) of other illicit drugs complicates any interpretation of data obtained. The second reason that results are controversial is that with any human data relating to recreational drugs the results obtained are sometimes subject to emotional interpretation. There are those that believe that because a drug is used recreationally it is inherently dangerous and information gained should be put in the most negative light in order to "warn off" possible users. Such an approach naturally ignores the fact that any compound used obeys general pharmacological rules and adverse events relate to dose and frequency of use. At the other extreme there are those that feel that MDMA may have a possible therapeutic use or is a pleasant recreational drug with little propensity for producing long-term problems. Both these views sometimes appear to cloud any normal scientific rational discussion (see Green, 2004).

While, therefore, the possible long-term consequences of MDMA use remain a matter of sometimes rather ill-tempered debate, no one questions the fact that acute problems can arise from MDMA use, including an acute hyperthermic response that occasionally leads to a fatal outcome. In the UK approximately 15 young persons die every year following acute MDMA ingestion and the majority of these deaths can, to some extent, be attributed to the pathological consequences of severe hyperthermia. Both death and morbidity problems associated with the hyperthermia include: rhabdomyolysis, myoglobinuria and renal failure, liver damage and disseminated intravascular coagulopathy (see Kalant, 2001). These problems are a feature of heatstroke and have also been reported to occur after toxic doses of amphetamine, methamphetamine and 3,4-methylenedioxyethamphetamine (MDEA, 'eve') (Ginsberg et al., 1970; Kendrick et al., 1977; Tehan, 1993).

Despite the evidence of the clinical problems that are associated with the MDMA-induced hyperthermia, knowledge of the pharmacology of the response remains poorly understood despite the fact that exactly the same effect can be studied in experimental animals. The purpose of this review is to examine the acute physiology and pharmacology of MDMA-induced hyperthermia. The effect of prior MDMA-induced neurotoxic damage on the subsequent response of rats to a further dose of MDMA is also examined in order to discuss whether heavy recreational users of MDMA are likely to be at greater risk of an acute hyperthermic response when taking further recreational doses of the drug.

2. Acute consequences of MDMA administration on body temperature

2.1. Effect of MDMA on body temperature in humans

Hyperthermia is one of the major symptoms of acute MDMA-induced toxicity. Body temperatures in excess of 43 °C have been reported. Such changes may generally only occur following doses of MDMA greater than 1.5 mg/kg since Mas et al. (1999) did not observe a temperature increase following administration to volunteers of 125 mg (1.8 mg/kg in a person weighing 70 kg), while Liechti and Vollenweider (2000a) giving 1.5 mg/kg did detect a modest temperature rise. However, the conditions in which MDMA is taken may alter this value. MDMA is usually taken recreationally in dance clubs or 'raves' with hot, crowded rooms (both conditions leading to a larger elevation of MDMA-induced body temperature in animals; see Section 2.2) and by people engaged in very active dancing, which may also alter the response.

In addition to hyperthermia other, often fatal, clinical problems occur including disseminated intravascular coagulation (producing widespread bleeding and tissue necrosis), rhabdomyolysis, tachycardia, coagulopathy, myoglobinuria, thrombocytopenia, renal failure, oedema, acidosis and subarachnoid hemorrhage (Kalant, 2001, Green et al., 2003; Schifano, 2004). The pattern of changes is similar to heatstroke and it is likely that rhabdomyolysis, myoglobinuria, renal failure and liver damage are related to the severe elevation of body temperature (Kalant, 2001). Approximately 15 young persons die every year from acute MDMA-induced toxicity. It seems reasonable to propose that several

of these deaths each year could have been prevented if the hyperthermia could have been rapidly reversed. However, no selective antagonists have been reported. Citalopram is without effect on the hyperthermic response (Liechti and Vollenweider, 2000a) and the dopamine D2 receptor antagonist haloperidol also fails to antagonise the response (Liechti and Vollenweider, 2000b). These findings are generally consistent with preclinical data in rats, for while haloperidol did prevent MDMA-induced hyperthermia (Colado et al., 1998) the highly selective dopamine D2 receptor antagonist remoxipride did not (Mechan et al., 2002). Current treatment, therefore, relies on the use of dantrolene (Henry, 1992; Singarajah and Lavies, 1992; Tehan, 1993; Mallick and Bodenham, 1997; Hall et al., 1996), an approach that has been questioned (Barrett, 1992), or by trying to rapidly decrease body temperature by using ice packs.

Since experimental animals also experience a dose-dependent hyperthermia following MDMA it seems reasonable to use data obtained in preclinical studies to increase our understanding of the clinical problem and its treatment. The following sections, therefore, detail current knowledge on MDMA-induced hyperthermia in experimental animals.

2.2. MDMA-induced hyperthermia in rats

Rats housed in 'normal' room temperature conditions (20–22 °C) have been reported in many studies to have an acute dose-dependent hyperthermic response (Fig. 1) following administration of MDMA (Nash et al., 1988; Schmidt et al., 1990; Colado et al., 1993; Dafters, 1994; Broening et al., 1995; Malberg et al., 1996; O'Shea et al., 1998). This citation listing is not comprehensive and many other publications have reported similar findings. A couple of investigators have observed a hypothermic response (e.g. Marston et al., 1999) and this anomaly may be due to the ambient room temperature in which the animal is treated since this markedly influences the body temperature response. Higher ambient room temperature conditions result in rats having a hyperthermic response to MDMA, while lower ambient temperatures result in rats displaying a hypothermic response (Malberg and Seiden, 1998). This effect of ambient room temperature on the MDMA-induced temperature response of rats is well established. Dafters (1994) demonstrated that in rats housed at an ambient temperature of 11 °C MDMA produced a dose-dependent hypothermic response while at an ambient temperature of 24 °C a hyperthermic response occurred. A subsequent study by Dafters and Lynch (1998) observed MDMA-induced hyperthermia at an ambient temperature of 22°C and hypothermia at an ambient temperature of 17 °C, so the switching point occurs near the normal ambient room temperature in which rats are normally housed (20 °C). Broening et al. (1995) reported a hypothermic response at an ambient temperature of 10 °C when rats were 40 or 70 days old and hyperthermia if these rats were exposed to an ambient temperature of 25 °C or 33 °C. However, 10-day-old rats did not exhibit body temperature changes following MDMA administration.

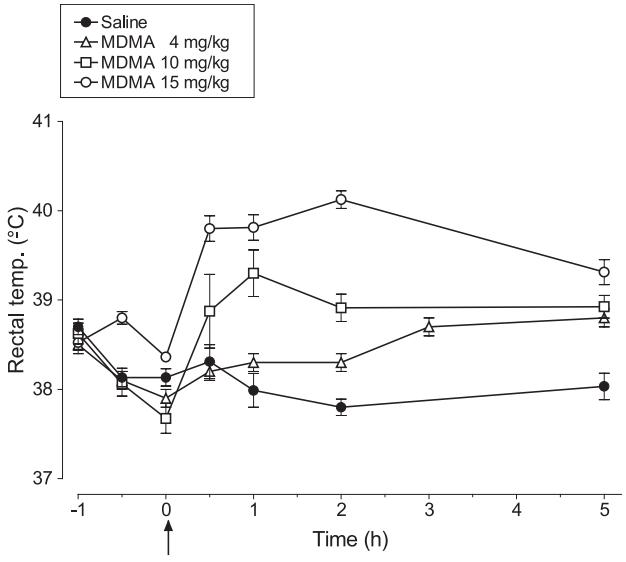

Fig. 1. Rectal temperature of rats injected with a single dose of MDMA (4, 10 and 15 mg/kg, i.p.) or saline. Results shown as mean and vertical lines indicate S.E.M. (n=6–9). MDMA produced a significant increase in temperature at the doses of 4 mg/kg [$F(1,13)=17.0$, $P<0.01$], 10 mg/kg [$F(1,15)=46.35$, $P<0.001$] and 15 mg/kg [$F(1,15)=189.35$, $P<0.001$] vs. saline. The hyperthermic response induced by MDMA 15 mg/kg was significantly larger than that induced by MDMA 10 mg/kg [$F(1,14)=19.47$, $P<0.001$] and MDMA 4 mg/kg [$F(1,12)=117.45$, $P<0.001$]. Similarly, MDMA 10 mg/kg vs. MDMA 4 mg/kg was significant [$F(1,12)=12.77$, $P<0.01$]. Reproduced from O'Shea et al. (1998) with permission of Elsevier.

Recently studies have been conducted to examine the effect of ambient temperature on the response of rats to 'binge' dosing; that is administering the drug several times over a short period of time to simulate the way the drug is sometimes taken recreationally (Weir, 2000; Parrott, 2002). At an ambient temperature of 19 °C repeated doses of MDMA produce an increasing peak response at higher doses and the peak is further increased at an ambient temperature of 30 °C (Green et al., 2004; Sanchez et al., 2004; Fig. 2).

What seems probable is that these results reflect in some way the mechanisms by which MDMA induces the body temperature change. A recent study by Mechan et al. (2002) demonstrated that the temperature of the rat tail was unaltered following a dose of MDMA that produced a significant rise in rectal temperature (Fig. 3). Since vasodilation of tail vessels is a major mechanism by which rats lose temperature (Grant, 1963) these results suggest that MDMA interferes with heat loss mechanisms and consequently the higher the ambient temperature the more impaired the ability to lose heat to the environment. This explanation is supported by the observation that the construction of the animal cage can alter the response. Rats housed on an acrylic cage floor have a larger hyperthermic response than those housed on a grid-like floor (Gordon and Fogelson, 1994). A similar mechanism of impaired heat loss has been proposed for methamphetamine-induced hyper-

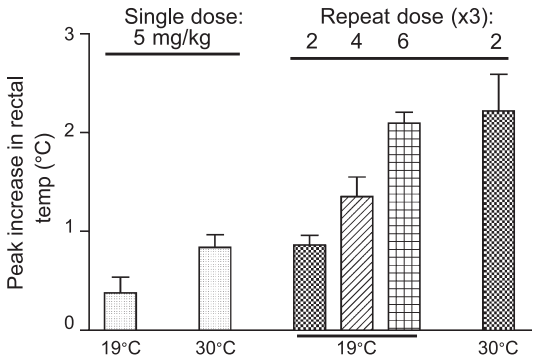

Fig. 2. The peak increase in rectal temperature after single or repeated doses of MDMA following subtraction of the appropriate saline-injected control value in control rats. The rats had been acclimatised to a room at an ambient temperature of 19 °C or 30 °C for 1 h before drug administration and were kept in that temperature for the duration of the study. Redrawn from data presented in Green et al. (2004) with permission of Springer-Verlag.

thermia (Mohaghegh et al., 1997). When the animal is in a low ambient temperature this loss of mechanism is presumably not important, although why the animal should actually lose heat is unclear unless the heat gain mechanism is also impaired.

Several years ago Gordon et al. (1991) examined other thermoregulatory mechanisms following an MDMA injection by measuring metabolic rate, evaporative water loss and rectal temperature of rats housed at an ambient temperature of 10, 20 and 30 °C. The responses were compared with those seen in control rats not injected with MDMA. The metabolic rate was increased in the MDMA-treated rats compared with that seen in control rats at an ambient temperature of both 20 and 30 °C. Evaporative water loss also increased in the MDMA-treated rats to a greater extent than control animals. In accord with other findings rats given MDMA showed a hypothermic response at an ambient temperature of 10 °C, no change at 20 °C and hyperthermia at an ambient temperature of 30 °C.

There is considerable evidence that MDMA administration produces a major release of both 5-HT and dopamine from their respective nerve endings in the forebrain (see Green et al., 2003). While there has been some assumption that the hyperthermia was associated with the increased 5-HT release (e.g. Shankaran and Gudelsky, 1999), recent studies have indicated that it is dopamine release that is the primary mechanism. Administration of the 5-HT uptake inhibitor fluoxetine produces a major inhibition of MDMA-induced 5-HT release but has no effect on the hyperthermic response in the same animals (Mechan et al., 2002) confirming findings in separate groups of animals (Schmidt et al., 1990; Berger et al., 1992; Malberg et al., 1996). Furthermore, most 5-HT receptor antagonists and the dopamine D2 receptor antagonist remoxipride failed to antagonise MDMA-induced hyperthermia (Mechan et al., 2002). However the dopamine D1 receptor antagonist *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzapine (SCH23390) was an effective antagonist of the hyperthermic response.

This indication that dopamine D1 receptors are involved in the hyperthermia induced by amphetamine derivatives is supported by the evidence that the hyperthermia resulting from administration of the 5-HT releasing drug *p*-chloroamphetamine is unaltered by fluoxetine administration or the 5-HT depleting drug *p*-chlorophenylalanine (Sugimoto et al., 2001).

While specific evidence that rats, as opposed to mice, are susceptible to 'aggregation toxicity' (see Section 2.3) following MDMA does not exist, it is reasonable to suppose that such a phenomenon may occur in rats given the evidence that impaired heat loss occurs after MDMA administration and that water deprivation enhances the hyperthermic response (Dafters, 1995).

2.3. MDMA-induced hyperthermia in mice and guinea pigs

The temperature response which follows MDMA administration in mice appears to be more variable than that seen in rats and has generally been examined during administration of multiple doses of the drug. Both the strain of mouse and dose appear to influence the size and direction of the response detected.

C57BL/6J mice display hyperthermia after repetitive dosing (20 mg/kg) with MDMA (Johnson et al., 2000, 2002b; Miller and O'Callaghan, 1994). MDMA also produces a dose-dependent increase in body temperature in Charles River mice following a single dose of 5, 10 and

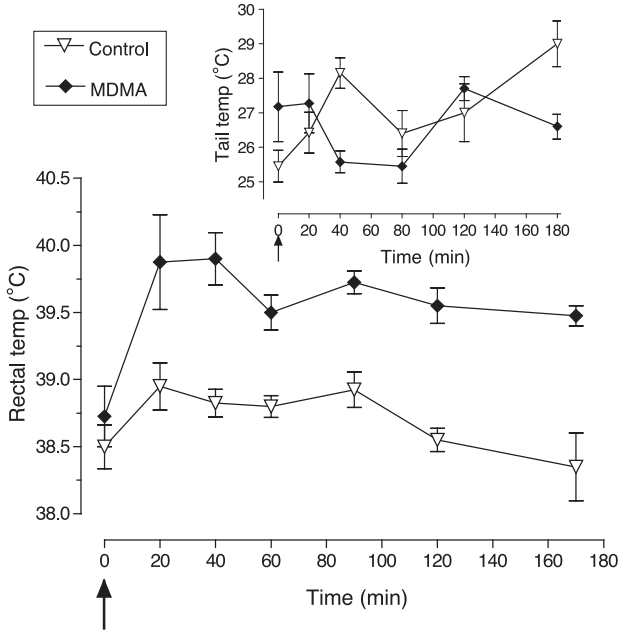

Fig. 3. Effect of MDMA on temperature. Main graph: rectal temperature of rats injected with MDMA (12.5 mg/kg, i.p.) or saline at t=0. MDMA produced a significant rise in body temperature ($F(1,6)=61.17$, $P<0.001$) during $t_{20–170}$. Inset graph: tail skin temperature of rats injected with MDMA (12.5 mg/kg, i.p.) or saline at t=0. MDMA had no significant effect on tail skin temperature compared to control animals. Reproduced form Mechan et al. (2002) with permission of the British Pharmacological Society and Stockton Press.

20 mg/kg (Carvalho et al., 2002). In contrast, MDMA produces dose-dependent hypothermia in BALB/c mice which was very long lasting (Johnson et al., 2002a). Swiss-Webster mice have a biphasic response to repeated doses of MDMA, hypothermia being the predominant effect following repeated doses of 10 mg/kg while hyperthermia followed by hypothermia being seen after repeat doses of 30 mg/kg (O'Shea et al., 2001). We observed only dose-dependent hyperthermia when NIH/Swiss strain mice were injected with repeated doses of MDMA (Colado et al., 2001) and recently reported a similar response in C57BL/6J mice (Sanchez et al., 2003; Fig. 4). The mechanism by which MDMA produces these changes in temperature in mice appears not to involve the acute dopaminergic effects of the drug since pre-treatment with 1-[2-*bis* (4-fluorophenyl) methoxy]ethyl]-4-3-phenylpropyl]piperazine (GBR 12909), a specific dopamine uptake inhibitor, did not alter the MDMA-induced temperature response of Swiss-Webster mice (O'Shea et al., 2001). The possible role of 5-HT is less clear since the acute effects of the drug on 5-HT levels in the brain were similar at all doses tested and did not follow the pattern exhibited by the temperature response, but pretreatment with fluoxetine did prevent the hyperthermia induced by MDMA.

The fact that amphetamine produced a greater acute toxic response in aggregated (grouped) mice than those housed singly was first observed by Gunn and Gurd (1940). This observation was extended by Chance (1946) who reported that elevated ambient temperature, poor hydration and loud noise also increased toxicity. While raised body temperature appears to be the primary cause of the acute toxic response (Askew, 1961; Craig and Kupferberg, 1972) it does not seem to be the sole mechanism (Wolf and Bunce, 1973). More recently Morton et al. (2001) extended these findings to methamphetamine while Fantegrossi et al. (2003) recently reported that the 'aggregation toxicity' response also occurs after MDMA injection.

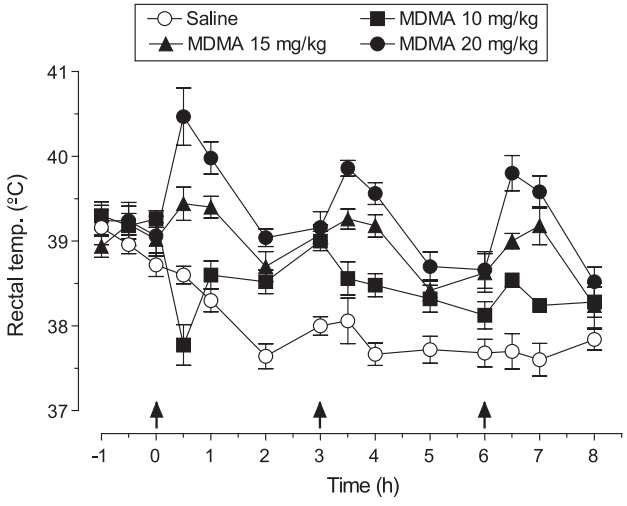

Fig. 4. Rectal temperature of C57BL/6J mice injected with repeated doses of MDMA (10, 15 and 20 mg/kg, i.p.) or saline. Mice received 3 injections at 3 h interval. Results shown as mean and vertical lines indicate S.E.M., (n=5). There was no difference in basal temperature of the groups ($F(3,16)=0.908$, n.s.). MDMA produced a significant increase in temperature at doses of 10 ($F(1,8)=35.23$, $P<0.001$), 15 ($F(1,8)=135.87$, $P<0.001$) and 20 mg/kg ($F(1,8)=207.14$, $P<0.001$) vs. saline. The hyperthermic response induced by 20 mg/kg MDMA was significantly larger than that induced by 15 mg/kg MDMA [$F(1,8)=28.68$, $P<0.001$] and 10 mg/kg MDMA [$F(1,8)=163.73$, $P<0.001$]. Similarly, 15 mg/kg MDMA vs. 10 mg/kg MDMA was significant [$F(1,8)=71.53$, $P<0.001$].

Only one study appears to have been performed on the MDMA-induced temperature response in guinea pigs. In this, guinea pigs were injected with MDMA (20 mg/kg) twice daily for 3 days. Only the first injection produced hyperthermia, there being no significant temperature response after the second dose on day 1 or the fifth dose on day 3 (Saadat et al., 2004).

3. Effect of a prior MDMA-induced lesion on the temperature response of rats to a subsequent dose of MDMA

Since MDMA is often ingested in crowded and hot dance club conditions, studies have been made on not only the effect of MDMA administration on the temperature response of rats when housed in high ambient temperature but also the effect of a prior MDMA-induced lesion. While unequivocal evidence for heavy or regular MDMA use producing neurotoxic damage in the human brain is still not available, there are substantial data which indicate that changes in cerebral function have occurred in the brain of some MDMA users (see Green et al., 2003). Therefore, it is reasonable to mimic possible damage by giving a neurotoxic dose of MDMA to rats and then examine the consequences to the ability of the animals to thermoregulate.

The first report that a prior MDMA-induced lesion altered the ability of an animal to thermoregulate was that of Dafters and Lynch (1998) who found that lesioned animals showed a sustained hyperthermic response (compared to control animals) following exposure to a 60-min period of high ambient temperature. This observation was confirmed by Mechan et al. (2001) who observed a more rapid increase in body temperature when the rats were exposed to high temperature and a slower decrease towards normal values than control animals when they were returned to a normal ambient temperature (Fig. 5).

Several studies have been conducted which have examined whether a prior neurotoxic dose of MDMA alters the temperature response of rats given a subsequent dose of the drug. However, the data obtained has been somewhat contradictory. Dafters (1995) gave daily doses of MDMA (7.5 mg/kg) on 14 consecutive days and reported a gradual enhancement of the peak temperature over time. This observation is difficult to interpret as it is not known whether this dose regime induced neurotoxic damage to 5-HT nerve endings. What might well be happening is that

there was a sustained loss of 5-HT content due to the releasing properties of MDMA which in turn produced an impaired ability of the rats to lose heat (see later). Shankaran and Gudelsky (1999) gave rats a neurotoxic dose regime of MDMA which produced a 45% loss of striatal 5-HT content and then examined the response of these animals to a challenge dose of MDMA (7.5, 10 and 20 mg/kg) 7 days later. A dose-dependent inhibition of the hyperthermic response was seen in the lesioned rats, although the attenuation of the response at the highest dose was not statistically significant.

The recent results of Green et al. (2004) are in disagreement with those of Shankaran and Gudelsky (1999) in that rats given a neurotoxic dose of MDMA (12.5 mg/kg) showed a similar response to the control animals when a dose of MDMA (5 mg/kg) was given to the animals when housed at an ambient temperature of 19 °C. Beveridge et al. (2004) also failed to find an altered response, compared with control animals, when rats given this neurotoxic dose of MDMA were challenged with MDMA (12.5 mg/kg). However, this lesioning dose of MDMA (12.5 mg/kg) only produces a cerebral 5-HT loss of around 30% (Mechan et al., 2001, 2002), which is much more modest than that produced in the study of Shankaran and Gudelsky (1999). When MDMA (12.5 mg/kg)-lesioned rats were challenged with MDMA (5 mg/kg) when housed at an ambient temperature of 30 °C their hyperthermic response was larger than control animals and the return towards pre-treatment values was slower (Green et al., 2004; Fig. 6). When repeated 'binge' doses of MDMA (2 mg/kg, three times at 3 h intervals) were given to prior lesioned animals housed at 30 °C there was a greater increase in rectal temperature than that seen in control animals following the first two injections, but a similar response after the third

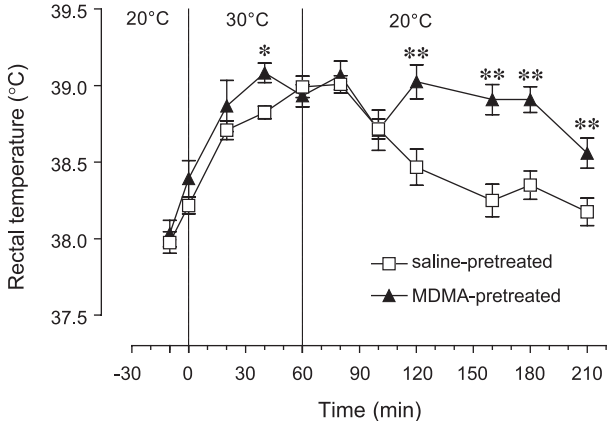

Fig. 5. Effect of MDMA-pretreatment on rectal temperature, both during and following exposure to a high ambient temperature (30.0±0.5 °C) for 60 min. Pretreatment values were: control group, 38.0±0.1 °C (n=12) and MDMA-pretreated group, 38.0±0.1 °C (n=12). MDMA treated group different from control group at $t_{0–60}$: $F(1,44)=8.78$, $P=0.005$ and at $t_{60–210}$: $F(1,77)=72.2$, $P=0.0001$. Different from control group: *p=0.01–0.05; **p<0.001. Reproduced form Mechan et al. (2001) with permission of Springer-Verlag.

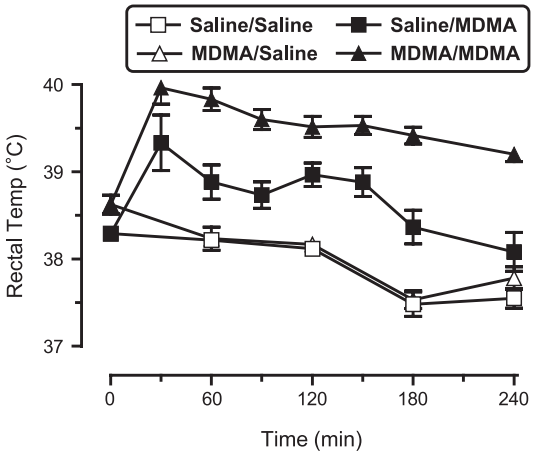

Fig. 6. The acute temperature response at an ambient temperature of 30 °C of rats administered saline 5 weeks after an injection of saline (□) or a neurotoxic dose of MDMA, 12.5 mg/kg (△) and rats administered a challenge dose of MDMA (5 mg/kg) 5 weeks after saline (■) or a neurotoxic dose of MDMA, 12.5 mg/kg (▲). Reproduced form Green et al. (2004) with permission of Springer-Verlag.

injection when the peak response is maximal (Green et al., 2004). Presumably by the third injection of MDMA the concentration of 5-HT in the brain of the control animals has been acutely depleted to that present in the lesioned animals and their temperature response is, therefore, similar to that seen in lesioned rats (Green et al., 2004).

Overall, these data suggest that fully functional 5-HT mechanisms are required to allow body temperature loss in high ambient temperature conditions. Two other studies support this interpretation. Giacchino et al. (1983) found that rats exposed to an ambient temperature of 32 °C had an increased tail temperature, presumably due to vasodilatation to assist body temperature loss. Pretreatment with the 5-HT depleting drug p-chlorophenylalanine resulted in rats exposed to an ambient temperature of 32 °C having a smaller temperature rise, implicating 5-HT in the thermoregulatory mechanism.

Similarly we have recently observed that p-chlorophenylalanine pretreatment results in rats having a greater and more sustained rectal temperature rise following MDMA administration than saline-injected control animals (Saadat, O'Shea, Elliott, Colado and Green, unpublished). Overall, the data suggest that decreased 5-HT function impairs the ability of the rat to lose heat when it is housed at high ambient temperature.

4. Role of cytokines in the MDMA-induced hyperthermic response

It is well established that cytokines such as interleukin-1β, interleukin-6 and tumour necrosis factor-α increase body temperature acting by direct or indirect mechanisms on the brain and, in particular, interleukin-1β has been shown to be involved in the development of the hyper-

thermic response induced by exogenous pyrogens such as lipopolysaccharide (Klir et al., 1994), turpentine (Luheshi et al., 1997) or leptin (Luheshi et al., 1999). Administration of interleukin-1β antibodies or an interleukin-1 receptor antagonist to experimental animals inhibits the rise in temperature induced by these external inflammatory stimuli.

Recently we have shown that immediately following MDMA administration to rats there is an acute increase in interleukin-1β concentration in the hypothalamus and cortex. The up-regulation of interleukin-1β production appears at an early time-point after MDMA and is of short duration, levels returning to basal values 12 h after drug injection (Orio et al., 2004).

Interestingly, there was a clear dissociation in the time course of the changes induced by MDMA on body temperature and interleukin-1β release. While a marked hyperthermia is evident within the first 20 min of MDMA administration (Colado et al., 1993), with rectal temperature peaking 60 min after treatment and remaining elevated for over 12 h, the increase in levels of interleukin-1β peaked at 3 h (Orio et al., 2004). These data, and the more definitive observation that intracerbroventricular administration of interleukin-1 receptor antibody did not modify the peak hyperthermic response immediately following MDMA, indicate that interleukin-1β production could be a consequence of, rather than the cause of, hyperthermia and that hyperthermia could represent a signal generated by MDMA which occurs early enough to allow secretion of interleukin-1β and probably a host of other soluble factors from the microglia. In line with these results we found that when animals are kept at an ambient temperature of 4 °C during MDMA treatment the hyperthermic response is totally abolished and there is a significant reduction in interleukin-1β production. These data indicate that release of interleukin-1β is, in part, a consequence of the hyperthermia (Orio et al., 2004).

In addition to increasing interleukin-1β release, MDMA also subsequently induced an increase in the density of peripheral benzodiazepine receptor binding sites, labelled with [^{3}H]PK11195 ([(1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)3-isoquinolinecarboxamide)], in hypothalamus and cortex of the rat. This parameter could be reflecting an activation of microglia as revealed by immunohistochemical studies in the anterior hypothalamus. However, and in contrast to the increase in interleukin-1β release, neither the up-regulation of peripheral benzodiazepine receptor binding sites nor the staining for OX-42, which stains activated microglia, were significantly modified when the hyperthermic response to MDMA was abolished. These data, together with the fact that the maximal microglial activation occurs 24 h after MDMA (when the hyperthermia induced by MDMA has disappeared), indicate that a hyperthermic response may not be necessary for the morphological changes that occur in the microglia.

MDMA, as occurs with other neurotoxins which also cause hyperthermia such as lipopolysaccharide, not only induces a rise in interleukin-1β levels in the hypothalamus, the primary site of thermoregulation in the brain (Kluger, 1991) but also in other extrahypothalamic regions such as cortex (Quan et al., 1994; Nguyen et al., 1998; Orio et al., 2004). It is worth mentioning that the hypothalamus is not the only brain region involved in thermoregulatory control and receptors for interleukin-1β have been identified in the cortex and hippocampus (Farrar et al., 1987; Yabuuchi et al., 1994; Gayle et al., 1997). In addition, injection of interleukin-1β into the striatum (Grundy et al., 1998) produces an increase in the rectal temperature of rats and injection of interleukin-1 receptor antibody into the hippocampus significantly attenuates lipopolysaccharide-induced fever (Cartmell et al., 1999).

5. Locomotor activity and the hyperthermic response

In addition to hyperthermia, MDMA administration also produces a dose-dependent increase in spontaneous locomotor activity. This behavioural change is observed immediately after the administration of the drug (Dafters, 1994, 1995). It has been suggested that the changes in body temperature observed after MDMA administration might be the result of the increased activity, however, studies have shown a dissociation between MDMA-induced hyperthermia and hyperlocomotion. Dafters (1994) observed no change in the hyperlocomotion of MDMA-treated rats kept at a low temperature (11 °C) before (for 24 h) and/or after treatment (immediately following injection) compared with those treated and maintained at standard room temperature (24 °C), while the hyperthermic response was prevented. At high ambient temperature, a condition in which the hyperthermic response to the drug is potentiated, the hyperkinetic effect remained unchanged (Dafters, 1995). Further evidence for the lack of association between the hyperthermic and hyperkinetic effects induced by MDMA comes from the fact that a low dose of MDMA (5 mg/kg) which causes a significant increase in locomotor activity at standard room temperature (20 °C) fails to cause hyperthermia. At an elevated room temperature (30 °C), the same dose produced a marked hyperthermia but did not alter the absolute locomotor response compared with that of rats given MDMA at standard room temperature (O'Shea, Orio, Escobedo, Sanchez, Navarro, Green, Colado, unpublished observations).

6. Hyperthermia and subsequent neurotoxicity

The role and importance of the hyperthermic response in MDMA-induced neurotoxicity in the rat has been the subject of much study. The degree of long-term damage produced by MDMA appears to be closely related to the

magnitude of the hyperthermic response and a close correlation has been found between the temperature response and the degree of neurotoxicity (Malberg and Seiden, 1998). Further evidence stems from studies at different ambient temperatures. Maintenance of animals at low ambient temperatures (10 °C) before and/or after treatment with MDMA prevents the hyperthermic response, producing hypothermia in some cases, and either attenuates or eliminates the MDMA-induced neurotoxicity (Schmidt et al., 1990; Broening et al., 1995). At elevated room temperatures (26–33 °C), both the hyperthermic response and the neurotoxicity are potentiated (Broening et al., 1995, Sanchez et al., 2004).

Despite this close relationship between the acute hyperthermia and subsequent neurotoxicity the exact role of hyperthermia in the development of the neurotoxic response is difficult to define since it is possible to observe neurotoxicity in the absence of hyperthermia. Multiple doses of low-dose MDMA (4 mg/kg, twice daily for 4 days) produced a substantial neurotoxic effect in Dark Agouti rats but caused only a slight increase in temperature above saline-treated controls after the first dose. This increase in temperature after the initial dose was not sufficient in itself to produce a neurotoxic effect (O'Shea et al., 1998). In addition, high doses of MDMA have also been reported to produce serotonergic toxicity in the absence of a hyperthermic response (Broening et al., 1995).

Further evidence of the role of hyperthermia in MDMA-induced neurotoxicity derives from studies of various putative protective agents. In early studies, before the importance of the hyperthermic response in neurotoxicity had been fully recognized, various compounds were ascribed a neuroprotective role which was later found to be due to their prevention of the acute hyperthermic response and in some cases their producing frank hypothermia (Farfel et al., 1992; Hewitt and Green, 1994). Thus, compounds such as haloperidol, which prevents MDMA-induced hyperthermia (Colado et al., 1999a), pentobarbitone, *N*-methyl-D-aspartate antagonists such as dizocilpine, or the 5-HT_2 receptor antagonist ketanserin which produce overt hypothermia (Farfel and Seiden, 1995; Malberg et al., 1996; Colado et al., 1999b) all appear to have no specific protective effect against MDMA-induced neurotoxicity since their protective action was removed when the temperature of the animals was kept similar to that in animals treated with just MDMA.

On the other hand, a few agents have been found which protect against the neurotoxicity of MDMA with no modification in temperature. For example α-phenyl-*N*-tert-butyl nitrone, a free radical trapping nitrone, partially prevented the MDMA-induced reduction in [^{3}H]paroxetine binding without modifying the hyperthermic response to the drug (Colado et al., 1997). The efficacy of α-phenyl-*N*-tert-butyl nitrone supports other evidence for the involvement of free radicals in MDMA-induced damage (see Green et al., 2003) and this helps clarify the role of body temperature in the neurodegenerative process. Free radical formation in the brain following MDMA administration is markedly enhanced in hyperthermic animals (Colado et al., 1998) and links with evidence that in ischemia-induced damage, free radical formation is influenced by body temperature, lowering body temperature resulting in decreased free radical formation (Globus et al., 1995; Kil et al., 1996).

Clomethiazole also appears to possess a partial protective action which is independent of its temperature lowering effects (Colado et al., 1999c). Fluoxetine, a selective 5-HT reuptake inhibitor, completely prevents the neurodegenerative effects of MDMA and also fails to alter the increase in temperature produced by the drug (Sanchez et al., 2001).

Taken together, this evidence indicates that hyperthermia has an important modulatory role but is not an essential factor in the neurotoxicity induced by the drug. Other factors such as high doses or increased frequency of dosing are also important factors and may overcome a lack of hyperthermic response to produce neurotoxicity.

7. Conclusions

MDMA-induced hyperthermia is a serious physiological event not only because it can produce severe adverse consequences in humans as well as experimental animals but also because it plays a major role in determining the severity of the long-term MDMA-induced neurotoxicity when it occurs. The pharmacology of the hyperthermic response is steadily becoming defined and the influence of the environmental conditions present when the drug is ingested is also now well characterized. Such knowledge should assist in deciding how to treat persons who are suffering from MDMA-induced hyperthermia and also determines how to take steps to minimise the occurrence of this adverse event, given that we must assume that young people will continue to use the drug recreationally.

Note added in proof

Sprague et al. (Br. J. Pharmacol. 142, 667–670, 2004) have recently extended their earlier findings and demonstrated the involvement of α_1- and β_3-adrenoceptors in the acute thermogenic effect of MDMA.

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