BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), 160, 1029–1044

0007-1188/10
www.brjpharmacol.org

REVIEW

The role of monoamines in the changes in body temperature induced by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and its derivatives

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Hyperthermia is probably the most widely known acute adverse event that can follow ingestion of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) by recreational users. The effect of MDMA on body temperature is complex because the drug has actions on all three major monoamine neurotransmitters [5-hydroxytryptamine (5-HT), dopamine and noradrenaline], both by amine release and by direct receptor activation. Hyperthermia and hypothermia can be induced in laboratory animals by MDMA, depending on the ambient temperature, and involve both central thermoregulation and peripheral changes in blood flow and thermogenesis. Acute 5-HT release is not directly responsible for hyperthermia, but 5-HT receptors are involved in modulating the hyperthermic response. Impairing 5-HT function with a neurotoxic dose of MDMA or *p*-chlorophenylalanine alters the subsequent MDMA-induced hyperthermic response. MDMA also releases dopamine, and evidence suggests that this transmitter is involved in both the hyperthermic and hypothermic effects of MDMA in rats. The noradrenergic system is also involved in the hyperthermic response to MDMA. MDMA activates central α_{2A}-adrenoceptors and peripheral α_1-adrenoceptors to produce cutaneous vasoconstriction to restrict heat loss, and β_3-adrenoceptors in brown adipose tissue to increase heat generation. The hyperthermia occurring in recreational users of MDMA can be fatal, but data reviewed here indicate that it is unlikely that any single pharmaceutical agent will be effective in reversing the hyperthermia, so careful body cooling remains the principal clinical approach. Crucially, educating recreational users about the potential dangers of hyperthermia and the control of ambient temperature should remain key approaches to prevent this potentially fatal problem.
British Journal of Pharmacology (2010) **160**, 1029–1044; doi:10.1111/j.1476-5381.2010.00722.x

Keywords: 3,4-methylenedioxymethamphetamine; 3,4-methylenedioxyethamphetamine; 3,4-methylenedioxyamphetamine; hyperthermia; hypothermia; 5-hydroxytrypamine; dopamine; noradrenaline; thermoregulation

Abbreviations: 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; BRL44408, 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole; DA, dark agouti; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; GBR 12909, 1-[2-[*bis*-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride; *m*-CPP, *m*-chlorophenylpiperazine; MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxyethamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MK-212, 6-chloro-2(1-piperazinyl)-pyrazine; PCPA, *p*-chlorophenylalanine; SCH23390, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; SR59230A, 1-(2-ethylphenoxy)-3-[[(1*S*)-1,2,3,4-tetrahydro-1-naphthalenyl]amino]-(2*S*)-2-propanol hydrochloride; WAY100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide

Introduction

Hyperthermia is an acute adverse event that can follow ingestion of 3,4-methylenedioxymethamphetamine (MDMA) by recreational users. The problem was first reported over 20 years ago as emergency rooms found they were admitting

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Received 2 December 2009; revised 22 January 2010; accepted 10 February 2010

young persons who had taken MDMA while they were present at dance clubs or parties, and especially at 'raves', an environment where the ambient temperature tends to be high and there is excessive physical exertion during dancing. These persons sometimes presented with a range of medical problems including rhabdomyolysis, myoglobinuria, renal failure, liver damage and disseminated intravascular coagulopathy. These are problems also seen in persons suffering from heatstroke (Kalant, 2001), and have also been reported to occur in persons who have taken high doses of other amphetamines such as methamphetamine, amphetamine and 3,4-methylenedioxyethamphetamine (MDEA or 'eve') (Ginsberg *et al*., 1970; Kendrick *et al*., 1977; Tehan, 1993). 3,4-Methylenedioxyamphetamine (MDA or 'love'), as well as being a drug of abuse in its own right, is a metabolite both of MDMA (de la Torre *et al*., 2000; Cole and Sumnall, 2003b) and MDEA (Ensslin *et al*., 1996).

Hyperthermia can also be induced in laboratory animals by administration of MDMA, and it has been associated with the long-term neurotoxic degeneration of 5-hydroxytryptamine (5-HT) nerve endings that can occur in the forebrain following administration of the drug. This association is indicated by the fact that the degree of neurotoxic damage is related to the severity of the hyperthermic response. While neurotoxicity can occur after MDMA in the absence of a hyperthermic response, there is nevertheless, in general, a close correlation between the temperature response and degree of neurotoxic damage (Malberg and Seiden, 1998). Furthermore, the damage induced by a neurotoxic dose of MDMA can be attenuated by placing the animals in a low ambient temperature (Schmidt *et al*., 1990; Broening *et al*., 1995), and exacerbated by placing the animals in a room with an elevated ambient temperature (Broening *et al*., 1995; Sanchez *et al*., 2004).

The ability of MDMA to induce neurotoxic damage in the brain of the recreational user remains contentious (Green, 2004). However, even if it does not occur there are good reasons to try and minimize the hyperthermia occurring in any recreational user because it is associated with other severe acute clinical problems and can be fatal. Current treatment relies on the use of dantrolene or trying to reduce the body temperature quickly by the use of ice packs.

Over the last few years, there have been a series of studies made in experimental animals to try and understand better the pharmacology of the hyperthermic response. This review focuses on the role of the major monoamine neurotransmitters (5-HT, dopamine and noradrenaline) in the body temperature altering the action of MDMA. Receptor nomenclature in this paper conforms to the *British Journal of Pharmacology*'s *Guide to Receptors and Channels* (Alexander *et al*., 2008).

Physiology of temperature control

Body temperature is closely regulated because changes in body temperature affect cellular function, and relatively small increases in body temperature are particularly dangerous in causing protein denaturation (Roti Roti, 2008) and pathological changes in nerve function (Sharma and Hoopes, 2003). Core body temperature is maintained as a balance between heat generation and heat loss. Increased heat generation and diminished heat loss help maintain body temperature in a cold environment. Heat production can be increased by increased skeletal muscle tone and activity, by shivering and by non-shivering thermogenesis (increase in cellular metabolism involving adrenergic and thyroid hormones), and heat loss is reduced by skin vasoconstriction, warm clothing and postural changes to reduce exposed surface area. In response to a warm environment, heat generation is decreased, and heat loss is increased by skin vasodilatation, sweating and removing clothes. The transfer of heat between the body and the environment involves four mechanisms: radiation (transfer of infrared radiation energy, from warmer to cooler objects); conduction (transfer of heat to objects, air or water, from warmer to cooler); convection (warm air currents rising from the skin to aid conduction, or by the effects of fans or wind); and evaporation (passive or obligatory evaporation in the airways and skin, and active evaporation by sweating). When the ambient temperature is above body temperature, heat loss relies on evaporation by sweating, but at high air humidity, sweat does not evaporate, and this may be of relevance to the 'rave' environment. Some differences between rodents and humans in thermoregulation should be noted. Rodent tail is a major organ of temperature regulation, so that increased tail blood flow and panting increase heat loss, whereas piloerection (to trap air for insulation) reduces heat loss, and the presence of brown fat allows more marked increases in heat generation than occurs in humans. Autonomic control of temperature involves mainly control of cutaneous blood flow, modulation of heat generation and sweating.

MDMA-induced temperature changes in rats

Many studies have reported that when rats are housed in normal ambient room temperatures (20–22°C), their body temperature is elevated by MDMA administration in a dose-dependent manner. This has been reported to occur in several strains by many investigators (see Green *et al*., 2003). However, a hypothermic response has also been reported to occur in rats housed at this temperature (Malberg and Seiden, 1998; Malpass *et al*., 1999; Daws *et al*., 2000; Bexis and Docherty, 2006). In the study of Bexis and Docherty (2006), both MDMA and MDEA produced only hypothermia in rats housed at 22°C, whereas MDA produced a transient hypothermia followed by hyperthermia.

At higher room temperatures (e.g. 30°C), hyperthermia is always seen, and hyperthermia induced in the rats is greater than that seen in rats housed in normal conditions (Green *et al*., 2005).

In contrast, when MDMA is given to rats housed in low-room temperature conditions, its effect is to lower body temperature. Dafters (1994) observed dose-dependent hypothermia in rats administered with MDMA when housed at 11°C, and subsequently reported hypothermia to occur when the rats were housed at 17°C (Dafters, 1995). Green *et al*. (2005) also observed hypothermia in rats housed at 15°C. This suggests that the switching point for inducing hypothermia

or hyperthermia occurs at a room temperature of around 19–22°C, which is not far below normal ambient conditions for rats. Broening *et al*. (1995) noted that rats aged 40 and 70 days showed hypothermia and hyperthermia, respectively, at low and high room temperatures, but that MDMA did not influence body temperature in 10 day rats. MDMA is not the only amphetamine compound to produce a temperature response that is dependent on ambient temperature. The simpler congener amphetamine also produces, respectively, hyperthermia and hypothermia when rats are housed, respectively, in warm or cool ambient temperature room conditions (Yehuda and Wurtman, 1972a,b).

MDMA-induced temperature changes in guinea pigs and mice

While MDMA does induce hyperthermia in guinea pigs, it does so only after the first dose, with a subsequent dose 6 h later having no effect. Further dosing at day 2 was also without effect in body temperature (Saadat *et al*., 2004).

Most investigations on MDMA-induced body temperature effects in mice have also generally used repeat dosing, and the responses obtained have indicated that the response obtained is very dependent on the strain being examined. MDMA induced dose-dependent hyperthermia in C57BL/6J (Miller and O'Callaghan, 1994, Johnson *et al*., 2000; 2002b; Sanchez *et al*., 2003; Bexis and Docherty, 2008), NIH/Swiss (Colado *et al*., 2001) and Charles River mice (Carvalho *et al*., 2002), but hypothermia in BALB/c mice (Johnson *et al*., 2002a). Swiss-Webster mice had a biphasic response to repeat doses of MDMA, with hypothermia being the major effect when 10 mg·kg^{-1} was injected, but hyperthermia followed by hypothermia being observed when doses of 30 mg·kg^{-1} were given (O'Shea *et al*., 2001). In C57BL/6J mice, a biphasic hypothermia followed by hyperthermia can be revealed by either α_2- or α_1-adrenoceptor blockade (Bexis and Docherty, 2005; 2008) (see below).

Effect of housing conditions on the temperature response of rodents to MDMA

Part of the explanation for these apparent contradictory results on the temperature response of rodents to MDMA when they are housed at *normal* ambient room temperature conditions (in addition to strain differences) is that the way they are housed influences the temperature response. It was observed many years ago that mice that were grouped together experienced a greater hyperthermic response following amphetamine than those housed singly (sometimes referred to as 'aggregation toxicity': Gunn and Gurd, 1940; Chance, 1946), and this phenomenon has been observed to occur in mice following MDMA (Fantegrossi *et al*., 2003). While the effect has not been formally reported to occur in rats, it is generally accepted by investigators to also occur in that species.

Even the cage housing can influence the hyperthermic response since Gordon and Fogelson (1994) observed a greater body temperature increase in rats given MDMA and housed in an acrylic floored cage than one with a grid type of flooring. This suggests that heat loss plays a major role in determining the body temperature following MDMA.

Effect of rat strain and MDMA metabolism on the MDMA-induced hyperthermic effect in rats

A recent study by Green *et al*. (2009) which used published data examined the relationship between dose administered and plasma concentration in rats, and found that data on the peak plasma concentration obtained from several rat strains could be pooled as there was no clear difference between values obtained at several dose values. This even included data obtained from dark agouti (DA) rats, although the data used were only those from male DA rats. Female DA rats are CYP2D6 deficient and are therefore considered a model of the poor metabolizer phenotype as they metabolize debrisoquine more slowly than either male DA rats or other rat strains (Al-Dabbagh *et al*., 1981). Because MDMA is demethylenated by CYP2D6 in humans (Tucker *et al*., 1994), the female DA rat has been examined as a model of the poor metabolizer phenotype. Following the same dose of MDMA to male and female DA rats, the females had higher peak temperature response and greater peak plasma MDMA concentration, suggesting that they did metabolize MDMA more slowly than males, and that it was MDMA that induces hyperthermia rather than a metabolite (Colado *et al*., 1995).

In humans, there is an increased gradient in the dose-versus-plasma-concentration plot as the dose increases, with a fourfold increase in plasma concentration with only a twofold increase in dose from 1 to 2 mg·kg^{-1}. This is because there is mechanism-based inhibition of MDMA metabolism, which means that MDMA inhibits CYP2D6, one of its major metabolizing enzymes (Tucker *et al*., 1994; de la Torre *et al*., 2000). This inhibition can occur within 1 h (Yang *et al*., 2006). Whether this inhibition also occurs in rats is less clear. Baumann *et al*. (2009) have recently published data on low-dose MDMA administration, which suggests auto-inhibition can take place. However, the study by Green *et al*. (2009) using data obtained by others over a wide dose range (and one encompassing dose given in many studies on hyperthermia) indicate any inhibition is modest as there was an approximately linear relationship between dose and plasma concentration of the drug. This is perhaps not surprising because rats metabolize debrisoquine (and therefore presumably also MDMA) using several P450 enzymes, including CYP2D1, in addition to CYP2D6 (Matsunaga *et al*., 1989).

A final point regarding 'translation' of rodent data to human events is that of dose. The data of Green *et al*. (2009) demonstrated that at low dose of 2 mg·kg^{-1} in humans produced a peak plasma concentration that was only achieved by giving approximately 7 mg·kg^{-1} to rats. This seems a reasonable projection given that a 100 mg MDMA dose (1.4 mg·kg^{-1}) provokes a 0.6°C oral temperature rise in humans (Farré *et al*., 2007), and a similar increase in rectal temperature is seen following an approximate fourfold higher i.p. dose (5 mg·kg^{-1}) to rats (Colado *et al*., 1995). Baumann *et al*. (2009)

reported similar plasma concentrations in both rats and humans at a dose of 1.4 mg·kg^{-1}. The data of Green *et al*. (2009) also indicated little difference at these low doses, but that the relationship broke down at higher doses due to mechanism-based inhibition of MDMA metabolism and the dose ratio required to produce similar plasma levels in rats to humans increased markedly. While a group of recreational users had median plasma concentrations in the region of those produced in rats by dosing at approximately 2 mg·kg^{-1} (see Irvine *et al*., 2006), some values were much higher, and replicating in rats the plasma concentration seen in humans suffering a severe toxic response may require dosing rats with up to five times that dose.

Role of 5-HT in the acute MDMA-induced hyperthermic response in rats and mice

Direct effect of MDMA-induced 5-HT release on hyperthermia

Because MDMA produces a major acute release of 5-HT from nerve endings in the brain, it has often been assumed that the hyperthermia results from the release of this amine (Shankaran and Gudelsky, 1999), particularly as administration of tryptophan and a monoamine oxidase inhibitor also markedly increases 5-HT release and induces hyperthermia (Grahame-Smith, 1971). However, there is now considerable evidence to suggest that 5-HT plays little or no role in the acute hyperthermic response. Pretreatment with a variety of selective and non-selective 5-HT receptor antagonists was found to have no effect on MDMA-induced hyperthermia (Mechan *et al*., 2002). It has also been observed that pretreatment with either of the 5-HT uptake inhibitors fluoxetine (Schmidt *et al*., 1990; Berger *et al*., 1992; Malberg *et al*., 1996) or citalopram (Piper *et al*., 2008) has no effect on MDMA-induced hyperthermia. Crucially, Mechan *et al*. (2002) showed with the use of *in vivo* microdialysis that fluoxetine almost totally inhibited MDMA-induced 5-HT release in the brain, but that the hyperthermic response in the same animals was the same as that seen in rats pretreated with saline. Recently, Rodsiri *et al*. (2008) also failed to find any correlation between the hyperthermic response and the increase in extracellular 5-HT in rats given low doses of MDMA.

The role of 5-HT in the temperature effects following MDMA administration to mice is less clear. In a study in Swiss-Webster mice by O'Shea *et al*. (2001), there seemed to be no correlation between the temperature change and the change in cerebral 5-HT concentration. However, fluoxetine did block the MDMA-induced hyperthermia.

Effect of 5-HT receptor function on the hyperthermic response

While microdialysis studies have failed to indicate that the hyperthermic effect of MDMA is the direct result of the acute release of 5-HT by the drug, there is substantial evidence to suggest that 5-HT receptor subtype function can modulate the temperature response. The problem, however, is interpreting the data, for several important reasons.

A major problem is the promiscuous nature of the 5-HT release on its receptors. It is reasonable to propose that released 5-HT is acting on many of its receptor subtypes including 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_3$ and 5-HT$_4$ (Gudelsky and Yamamoto, 2008). Agonists at several of these receptors are known to alter body temperature. For example, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) is normally hypothermic in both rats and mice (Goodwin and Green, 1985; Goodwin *et al*., 1985; Hjorth, 1985), although the effect is probably pre-synaptic in mice (Goodwin *et al*., 1985), but post-synaptic in rats (Bill *et al*., 1991). In contrast, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a selective 5-HT$_{2A}$ agonist, and *m*-chlorophenylpiperazine (*m*-CPP) and 6-chloro-2(1-piperazinyl)-pyrazine (MK-212), selective agonists of 5-HT$_{2C}$ receptors, induce hyperthermia (Gudelsky *et al*., 1986; Aulakh *et al*., 1995; Mazzola-Pomietto *et al*., 1997). While there have been several studies that have attempted to dissect the receptors responsible for inducing the temperature changes following MDMA, absolute conclusions are difficult due to the lack of total receptor subtype selectivity of any antagonist used. For example, while ketanserin is an effective 5-HT$_{2A}$ antagonist, it is also an effective adrenoceptor antagonist. Consequently, while several reports have implicated 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors as having a role in inducing MDMA-induced temperature changes (Gudelsky *et al*., 1986; Mechan *et al*., 2002; Herin *et al*., 2005; Gudelsky and Yamamoto, 2007), an action via adrenoceptors cannot be discounted.

A further complication is that alterations in 5-HT function can influence dopamine release, and if, as suggested below, dopamine release has a key role in inducing both hypothermia and hyperthermia, then 5-HT being released onto its receptors will influence the temperature changes indirectly. However, the way that 5-HT and dopamine can interact is clearly brain region specific. For example, Leggio *et al*. (2009) reported that prefrontal cortex 5-HT$_{2C}$ receptors facilitate dopamine release in the accumbens, while both Di Matteo *et al*. (2008) and Alex and Pehek (2007) suggest that stimulation of most 5-HT receptor subtypes generally induces dopamine release, although the 5-HT$_{2C}$ receptor mediates an inhibitory effect on release.

One group of studies that does provide clear data on the role of 5-HT receptor subtypes on induction of MDMA-induced temperature changes are those on thermogenesis. Thermogenesis in brown adipose tissue has been shown to be increased by DOI, the 5-HT$_{2A}$ receptor agonist, but decreased by 8-OH-DPAT the 5-HT$_{1A}$ receptor agonist, thereby influencing heat production (Ootsuka and Blessing, 2006). The same group subsequently found that MDMA administration to rats in a cool environment inhibited brown adipose tissue thermogenesis (and tail artery vasoconstriction), an effect antagonized by the 5-HT$_{1A}$ antagonist WAY 100635, thereby strongly implicating the 5-HT$_{1A}$ receptor in this response. The dopamine D$_2$ receptor antagonist potentiated the WAY100635 effect, indicating a role for dopamine (Rusyniak *et al*., 2008). The atypical neuroleptic clozapine, a drug which has antagonistic actions at several 5-HT receptor subtypes, as well as dopamine receptors, was also an effective compound in reversing MDMA-induced hyperthermia and peripheral vasoconstriction in rats and rabbits (Blessing *et al*., 2003), and reversed brown tissue thermogenesis induced by MDMA (Blessing *et al*., 2006).

However, in ligand binding studies, the 5-HT_{2A}-receptor antagonists clozapine and risperidone show high affinity for α_{1A}-adrenoceptors (pKi values of 8–9) and moderate affinity for α_{2A}-adrenoceptors (pKi values of 6–7) (Nasrallah, 2008), and similar affinities for clozapine but higher affinities for risperidone, were reported by Morimoto *et al*. (2002). Functionally, clozapine causes urinary incontinence by α_{1A}-adrenoceptor block (Fuller *et al*. 1996) and blocks α_2-adrenoceptor-mediated inhibition of renin release (Pettinger *et al*., 1976). The doses of clozapine (0.1–5 mg·kg^{-1}) and risperidone (0.5 mg·kg^{-1}) employed in the MDMA studies (Blessing *et al*., 2003; Shioda *et al*., 2008) should produce marked α_1-adrenoceptor antagonism, and this action should be considered as a possible mode of action in reversal of MDMA-induced cutaneous vasoconstriction.

Role of dopamine in the acute MDMA-induced hyperthermic response in rats and mice

MDMA not only produces a major acute release of 5-HT in the brain, it also induces a major release of the neurotransmitter dopamine (see Green *et al*., 2003), and there is now reasonable evidence to suggest that it is the release of this transmitter that is central to both the hyperthermic and hypothermic effects of MDMA in rats. In rats kept in normal ambient temperature conditions, the hyperthermic response following MDMA was unaffected by the dopamine D_2 receptor antagonist remoxipride, but was dose-dependently antagonized by the dopamine D_1 receptor antagonist SCH23390 (Mechan *et al*., 2002). In contrast, it was found that the MDMA-induced hypothermia was blocked by remoxipride, but not by SCH23390 in rats housed in cool ambient temperature conditions (Green *et al*., 2005).

The pharmacology of the hyperthermic and hypothermic response following MDMA may be indicative of 'warm' and 'cold' thermosensors (Bligh, 1979), responding preferentially to dopamine D_1 and D_2 receptors activated by MDMA-induced dopamine release, or the primacy of pre-synaptic and post-synaptic dopamine receptor function (Hjorth and Carlsson, 1987) at different ambient temperatures. This latter explanation is supported by the fact that dopamine agonists have opposite body temperature effects in normal rats and those pretreated with reserpine to inhibit pre-synaptic dopamine function (Verma and Kulkarni, 1993).

The involvement of dopamine in mice is not clear. Pretreatment with the selective dopamine uptake inhibitor GBR 12909 did not alter temperature response of mice to MDMA, suggesting that MDMA is not displacing dopamine from nerve endings to induce the hyperthermia (O'Shea *et al*., 2001).

Studies on binge dosing of MDMA and body temperature in rats

Over the last few years, the pattern of MDMA ingestion in humans has often involved repeated drug administration over a single short episode, which is referred to as 'binge dosing'

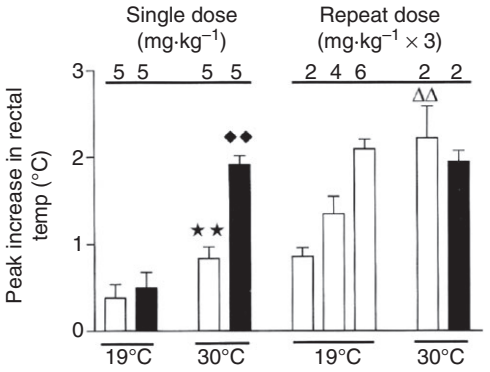

Figure 1 The peak increase in rectal temperature after repeated challenge doses of MDMA following subtraction of the appropriate saline-injected control value. The rats were treated with either saline (open bars) or a neurotoxic dose of MDMA (12.5 mg·kg^{-1}; closed bars) 5 weeks earlier. Challenge doses of MDMA (5 mg·kg^{-1}) were administered to rats housed at either 19 or 30°C as shown. ★★, different from saline-injected group at 19°C; $P < 0.01$; ◆◆, different from saline-injected group at 19°C, $P < 0.01$; rr, different from group given 2 mg·kg^{-1} when housed at 19°C, $P < 0.01$. Analysed by ANOVA plus Bonferroni correction for 't' test. Results shown as mean ± SEM with $n = 6$ per group. Reproduced from Green *et al*. (2004) with permission of Springer-Verlag.

(Hammersley *et al*., 1999; Topp *et al*., 1999; Winstock *et al*., 2001; Parrott, 2005). It is claimed that binge use of MDMA boosts its subjective effects and sustains the actions of the drug over time (Parrott, 2005). However, binge dosing is probably also employed by recreational users to divide the total dose and presumably therefore hopefully lessen the chance of an acute adverse event that could follow ingestion of a single high dose. Such hopes, however, fail to take account of the pharmacokinetics of the drug, particularly the half-life and metabolism of the compound.

Several studies have been conducted on the effects of binge-dosing schedules to rats that examined the body temperature response in animals housed in both normal and warm ambient room conditions. Repeated doses (three doses of 2, 4 or 6 mg·kg^{-1} given at 3 h intervals produced a linear ($r = 0.95$) dose-dependent increase in hyperthermia (Figure 1). The peak response seen after the third of the 2 mg·kg^{-1} doses (total dose 6 mg·kg^{-1}) was significantly greater than that seen after a single 5 mg·kg^{-1} dose, which suggests that binge dosing does not confer greater safety in terms of avoiding the hyperthermic response. The situation became progressively worse at higher doses (Green *et al*., 2004). When the rats were housed at 30°C, the situation worsened further because a single MDMA dose of 5 mg·kg^{-1} produced a greater hyperthermic response than that seen in animals housed at 20°C, but the peak response seen in animals treated with 3 × 2 mg·kg^{-1} was now more than twice that observed in rats given 5 mg·kg^{-1} when housed at 30°C, and over five times greater than that seen in rats housed at 20°C (Figure 1; Green *et al*., 2004).

These data suggest that humans who binge dose are not protecting themselves from the possibility of minimizing the chance of suffering an acute hyperthermic adverse event; indeed, bingeing may increase the possibility, particularly in warm room conditions. Pharmacokinetic studies suggest the problem of binge dosing may be even worse in humans than

in rats. The study of Green *et al*. (2009), using published data, examined the relationship between dose administered and plasma concentration in rats and humans. While there was an approximately linear relationship between dose and plasma concentration of the drug, in humans there was an increased gradient in the slope as the dose increases with a fourfold increase in plasma concentration with only a twofold increase in dose from 1 to 2 $mg \cdot kg^{-1}$ because of mechanism-based inhibition of MDMA metabolism (see earlier). Because evidence suggests that it is exposure to MDMA that produces the acute adverse event of hyperthermia, then this auto-inhibition could have severe consequences. Binge dosing produced an additive effect in terms of the peak hyperthermia (Green *et al*., 2004). In contrast, because the first dose of MDMA in humans inhibits metabolism within an hour (Yang *et al*., 2006), further dosing is likely to induce a greater than additive temperature response. Consequently, binge dosing experiments in rats may prove to be a poor model as they probably underestimate the potential problem in humans.

Effect of decreased brain 5-HT function on MDMA-induced hyperthermia

High or repeated doses of MDMA to rats result in selective long-term neurotoxic damage to 5-HT nerve endings in the forebrain (see Green *et al*., 2003). There is some evidence that similar changes may occur in the brains of some recreational users of the drug, but unequivocal evidence is lacking and opinions of investigators are divided (Green *et al*., 2003; Green, 2004; Gouzoulis-Mayfrank and Daumann, 2009; Selvaraj *et al*., 2009). It is beyond the scope of this review to examine such evidence. However, it is of interest to examine studies that have investigated the consequences of such a prior lesion on the hyperthermic response of rats following further ingestion of the drug, to determine what the effect of neurotoxicity might be. Such studies would also show what might be the effect any loss of brain 5-HT concentration induced by the MDMA-induced acute release of neuronal 5-HT and inhibition of tryptophan hydroxylase (see Green *et al*., 2003).

The first evidence that a loss of 5-HT in the brain resulted in impaired thermoregulation was the study of Dafters and Lynch (1998), which found that a prior neurotoxic dose of MDMA resulted in rats showing a sustained hyperthermic response following exposure to a 60 min period of high ambient room temperature when compared to the response seen in non-lesioned rats. This observation was confirmed and extended by Mechan *et al*. (2001) who reported that, when compared to non-lesioned rats, MDMA-lesioned animals displayed a more rapid rise in rectal temperature when placed in a high ambient temperature room, and a slower decrease to normal values when they were then placed back in a normal ambient temperature room. This suggested that heavy recreational users of MDMA might (if the drug does produce a sustained decrease in 5-HT function in the human brain) have a problem in thermoregulating in high ambient room temperature conditions. Consequently, further studies were conducted to see what effect further MDMA dosing had in animals with a prior neurotoxic lesion of 5-HT neurones.

Two studies found that a low dose of MDMA produced a similar hyperthermic response in rats previously given a neurotoxic dose of MDMA to that seen in saline-pretreated control animals when the rats were present in normal ambient room temperature conditions (Beveridge *et al*., 2004; Green *et al*., 2004). These results contrast with a study by Shankaran and Gudelsky (1999) who reported an inhibited hyperthermic response to the MDMA challenge dose in lesioned rats. However, the degree of 5-HT loss was greater (45%) in that study than that achieved in the studies of Beveridge *et al*. (2004) and Green *et al*. (2004). When the lesioned rats were given a subsequent dose of MDMA while housed in a warm room (30°C), the hyperthermic response was significantly greater than that seen in control rats, and the return to pretreatment values slower, consistent with the problems in thermoregulation seen by Mechan *et al*. (2001).

These data suggest that intact brain 5-HT function is required in rats for effective thermoregulation when they are present in warm conditions and challenged by a low dose of MDMA. This interpretation was strengthened by a later study which found that rats present in 30°C room conditions and pretreated with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA) to deplete the brain 5-HT concentration also had a prolonged hyperthermic response following injection of a low dose of MDMA. A similar prolongation of the response was seen following pretreatment with the non-selective 5-HT antagonist methysergide and the 5-HT_{1A} antagonist WAY100635 (Saadat *et al*., 2005). Giacchino *et al*. (1983) had previously shown that PCPA impaired the ability of rats to lose temperature in elevated temperature room conditions.

Together, these results suggest that a decrease in 5-HT function normally acting at 5-HT_{1A} receptors leads to an impaired ability of rats to lose heat in high ambient temperatures. This problem is expressed strongly when such animals are administered a hyperthermia-inducing dose of MDMA, and suggest that if heavy recreational users of MDMA may be a more risk than light users of experiencing a severe hyperthermic reaction, if they take the drug in hot conditions (such as a dance club).

The major mechanism by which rats lose heat is by vasodilation of the tail veins (Grant, 1963; Romanovsky *et al*., 2002). This leads to an increase in tail temperature. When rats are given MDMA, the rectal temperature rises, but the tail temperature does not (Mechan *et al*., 2002). This suggests that MDMA has interfered with this normal heat loss mechanism, probably by inducing peripheral vasoconstriction (Gordon *et al*., 1991; Pedersen and Blessing, 2001).

The results of both Dafters and Lynch (1998) and Mechan *et al*. (2001) outlined above suggested that rats with a prior neurotoxic lesion of 5-HT nerve endings induced by MDMA administration had a problem in losing body heat when exposed to high ambient room temperatures, and then returned to normal room conditions. This problem was also noted when lesioned rats were administered a dose of MDMA when present in warm room conditions, but not when housed at normal ambient temperature (Green *et al*., 2004). Again, it appears that the problem is associated with a defect in the functioning of the major heat loss organ in the rat, namely the tail; in this case, the effect of the prior neurotoxic

lesion. It is noteworthy that PCPA administration increases mortality in rats exposed to high ambient temperatures (Reid *et al*., 1968; Cronin, 1976) because this supports the notion that intact 5-HT function is required for effective heat loss mechanisms to occur in the rat. Consequently, administration of MDMA to rats with a prior neurotoxic lesion when housed at 30°C is faced with a double problem, impaired heat loss ability because of the acute effect of the MDMA, and impaired thermoregulation because of decreased 5-HT function in the brain. This is exactly what is seen when the tail temperature is examined (Green *et al*., 2005).

If heavy recreational users of MDMA do have impaired 5-HT functions in the brain and ingest further doses when present in hot rooms, then they may be more at risk of a hyperthermic crisis. However, humans, unlike rats, can cool themselves by shedding clothing. Nevertheless, in a possibly analogous situation, rats given MDMA and exposed to high ambient temperature conditions for 30 min then choose, when offered, a cooler environment in order to correct their hyperthermia (Jaehne *et al*., 2007). A prior neurotoxic lesion produced by repeated MDMA administration resulted in this cooler environment-seeking behaviour being disrupted (Jaehne *et al*., 2008). Finally, it should be noted that the human set point for thermoneutrality is at an ambient temperature of around 32°C (see for example. Srámek *et al*., 2000), which is far higher than the rat. These facts should mitigate against hyperthermia being a major problem for most human recreational users unless they are present in high ambient temperature room conditions.

Adrenergic mechanisms involved in MDMA-induced changes in body temperature

Although much of the research into the adverse actions of amphetamine derivatives has focused on 5-hydroxytryptaminergic and dopaminergic systems, there is also evidence for the involvement of the noradrenergic system, particularly in terms of peripheral actions.

Users of MDMA are reported to have elevated plasma catecholamine levels, which may be due to noradrenergic hyperactivity and may be linked to cardiovascular complications (Stuerenburg *et al*., 2002). The 'exchange diffusion model' was postulated to explain the combined uptake inhibition and neurotransmitter-releasing action of amphetamine derivatives (Fischer and Cho, 1979; Crespi *et al*., 1997). They are thought to compete with the neurotransmitter for the transporter, are transported into nerve terminals and the carrier functions in reverse to release neurotransmitter, although some results question a simple exchange diffusion hypothesis (Sitte *et al*., 2001). Hence, indirect release of neurotransmitters by amphetamine derivatives may be linked to their action at transporters. MDMA also inhibits MAO (Leonardi and Azmitia, 1994) to block metabolism of noradrenaline.

Actions at the monoamine transporters

MDMA was first reported to cause release of [^{3}H]5-HT from synaptosomes (Nichols *et al*., 1982). Battaglia *et al*. (1988) found that the affinity of MDMA for uptake sites was 5-HT > noradrenaline > dopamine, and affinities of MDA were comparable to those for MDMA. MDA also inhibits noradrenaline uptake with higher potency than dopamine uptake (Johnson *et al*., 1991).

The potency order in causing release of 5-HT in rat striatal slices was MDA > MDMA > MDEA (Schmidt, 1987), but the potency order in releasing dopamine from striatal slices (Schmidt, 1987) and synaptosomes (O'Loinsigh *et al*., 2001) was MDMA > MDA > MDEA.

MDMA also induces release of noradrenaline from striatal slices, and this is blocked by the noradrenaline transporter inhibitor desipramine (Fitzgerald and Reid, 1993). MDMA releases noradrenaline with a similar potency as for 5-HT release and a greater potency than for dopamine release (Johnson *et al*., 1991; Rothman *et al*., 2001). MDEA is less potent in inhibiting uptake of NA in the left ventricle when compared to MDA and MDMA (Cleary and Docherty, 2003). This may suggest an order of potency of MDA = MDMA > MDEA. Examination of Table 1 reveals that MDEA had the lowest affinity for all three transporters.

Actions at adrenoceptors

The amphetamine derivatives have been shown to have indirect sympathomimetic actions to potentiate the actions of noradrenaline (Fitzgerald and Reid, 1994; Al-Sahli *et al*., 2001; Cleary *et al*., 2002a) by competitive blockade of the noradrenaline transporter (Al-Sahli *et al*., 2001; Cleary and Docherty, 2003), and the linked ability to displace noradrenaline from peripheral noradrenergic nerve terminals (Fitzgerald

Table 1 Relative affinities/potencies of MDMA, MDA and MDEA at adrenoceptors and monoamine transporters

Site	*Response*	*Order of potency/affinity*	*Reference*
α1A	Binding	MDEA > MDA > MDMA	Bexis and Docherty, 2006
α1A	Contraction	MDA > MDMA > MDEA$^+$	Bexis and Docherty, 2006
α1D	Contraction	MDA > MDMA > MDEA$^+$	Bexis and Docherty, 2006
α2A	Pre-synaptic	MDMA > MDA > MDEA	Bexis and Docherty, 2006
α2A	Binding	MDMA > MDA > MDEA	Bexis and Docherty, 2006
SERT	5-HT release	MDA > MDMA > MDEA	Schmidt, 1987
DAT	DA release	MDMA > MDA > MDEA	Schmidt, 1987
NET	NA release	MDMA = MDA > MDEA	Cleary and Docherty, 2003

$^+$MDEA acts as partial agonist/antagonist.
Abbreviations: binding, ligand binding studies; DAT, dopamine transporter, NET, noradrenaline transporter; pre-synaptic, pre-synaptic/pre-junctional inhibition of nerve-evoked contraction; SERT, serotonin transporter.

and Reid, 1994; Lavelle *et al.*, 1999) and adrenal gland (O'Cain *et al.*, 2000), as well as direct receptor activation.

Effects of amphetamine derivatives at adrenoceptors *in vivo* may be a mixture of direct and indirect actions. Indirect actions would not be dependent on receptor subtype selectivity of the amphetamine derivative.

α_1-Adrenoceptors

In comparison to MDMA (and MDA), MDEA has significantly higher affinity for α_{1A}- and lower affinity for α_{2A}-adrenoceptors, but all had similar affinities at α_{2B}- and α_{2C}-adrenoceptors (Bexis and Docherty, 2006). However, despite this higher affinity for α_{1A}-adrenoceptors, MDEA had low potency at producing contractions of rat vas deferens (predominantly α_{1A}), suggesting low efficacy, and failed to contract rat aorta (predominantly α_{1D}), acting as an antagonist. Hence, the potency order for α_1-adrenoceptor agonism was MDA > MDMA > MDEA. (Bexis and Docherty, 2006) (see Table 1).

The major α_1-adrenoceptor-mediated actions involve vasoconstriction, causing a general rise in blood pressure and reducing cutaneous blood flow. In addition, coronary arterial contraction may increase the risk of myocardial infarction, as has been reported for cocaine (Mittleman *et al.*, 1999).

However, although MDMA has affinity for α_1-adrenoceptors, and produces contractions in tissues with α_1-adrenoceptors, there is evidence that at least some of these responses are resistant to α_1-adrenoceptor antagonists, and indeed other classical antagonists. Actions of MDMA and other amphetamine derivatives not involving adrenergic or 5-hydroxytryptaminergic receptors, may involve trace amine receptors (see Broadley, 2010).

In humans, MDMA, MDEA and MDA cause increases in arterial pressure (Gouzoulis *et al.*, 1993; Vollenweider *et al.*, 1998; Hegadoren *et al.*, 1999), and increases in both systolic and diastolic pressure occur in conscious rats (Bexis and Docherty, 2006). MDMA has been shown to have actions as an agonist at both α_1- and α_2-adrenoceptors, in addition to 5-HT_2 receptors, to raise blood pressure in the anaesthetized rat, and MDA produced larger pressor responses, but MDEA failed to raise blood pressure in anaesthetized (McDaid and Docherty, 2001) or conscious rats (Bexis and Docherty, 2006). The blood pressure actions of these agents in the rat are consistent with their actions at α1-adrenoceptors: MDA is more potent than MDMA as an agonist, and MDEA with low efficacy or acting as an antagonist. MDA has overall more marked cardiovascular, temperature and locomotor actions than the other agents (Bexis and Docherty, 2006). Urinary retention reported with MDMA (McCann *et al.*, 1996) may also involve peripheral α_1-adrenoceptor-mediated actions.

In the mouse, the α_1-adrenoceptor antagonist prazosin revealed an early significant hypothermia following MDMA, presumably because of blockade of vasoconstriction (Bexis and Docherty, 2008) (see Figure 2). In further studies, it has been demonstrated that both α_{1A}- and α_{1D}-adrenoceptors are involved in the initial hyperthermia to MDMA in mice, and combined block of both receptors is necessary to remove the full α_1-adrenoceptor-mediated hyperthermic component and reveal a hypothermia (Bexis and Docherty, 2008) (see Figure 2).

Indeed, peripheral effects of MDMA at α_1-adrenoceptors could explain a major component of its temperature actions, namely cutaneous vasoconstriction. Cutaneous vasoconstriction has been shown to contribute to the induction by MDMA of hyperthermia, although this has been previously reported to be largely mediated by central sympathetic activation involving serotonergic systems (Gordon *et al.*, 1991; Pedersen and Blessing, 2001; Blessing *et al.*, 2003). Such an effect may also explain the dependency of the temperature effects of MDMA on ambient temperature. At low ambient temperatures, cutaneous vasoconstriction is already marked so that MDMA produces little further vasoconstriction and early centrally mediated hypothermic actions of MDMA predominate. At high ambient temperatures, cutaneous dilatation has occurred; allowing a marked vasoconstrictor component to the actions of MDMA, and hyperthermia predominates. Hence, α-adrenoceptor-mediated peripheral vasoconstrictor actions of MDMA modulate central hypo- and hyperthermic components. In addition, there may be an α_1-adrenoceptor component of heat generation from brown fat.

α_2-Adrenoceptors

α_2-Adrenoceptors mediate pre- and post-synaptic actions of noradrenaline both in the central and peripheral nervous systems. α_2-Adrenoceptors have been separated into three

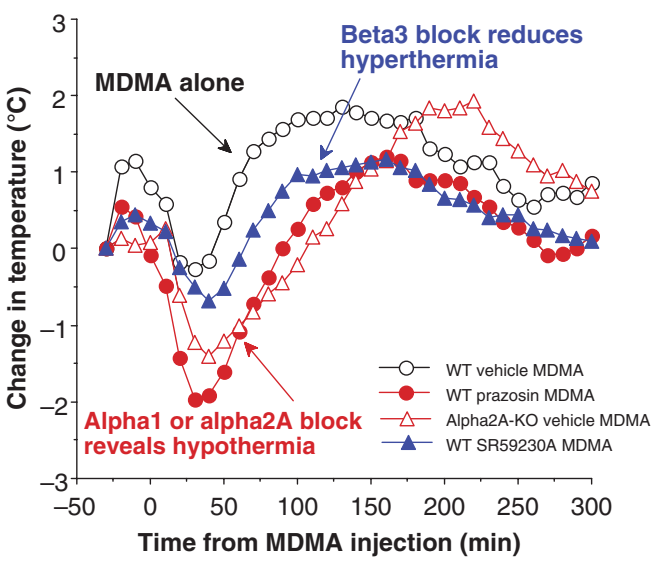

Figure 2 Summary of adrenergic components to the temperature response to MDMA in mice. The results shown are mean experimental data taken from Bexis and Docherty (2006; 2008; 2009), but for simplicity error bars have been omitted. Antagonist drugs or vehicle are injected 30 min before MDMA (20 $mg \cdot kg^{-1}$) injected at time zero. Knockout (or blockade) of α_{2A}-adrenoceptors, or blockade of α_1-adrenoceptors with prazosin, reveals an initial hypothermia to MDMA. Blockade of β_3-adrenoceptors with SR 59230A (low dose) reduces the maximum hyperthermia to MDMA. We interpret these results as follows: (i) α_1-adrenoceptor-mediated cutaneous vasoconstriction mediates a component of the hyperthermic response to MDMA, masking a hypothermic component; (ii) α_2-adrenoceptors in the CNS may be involved in this cutaneous vasoconstriction; and (iii) β_3-adrenoceptors (and possibly α_1-adrenoceptors) in adipose tissue mediate a component of heat generation.

subtypes, α_{2A}-, α_{2B} and α_{2C} (Bylund *et al*., 1994; Docherty, 1998), with α_{2A} and α_{2C} subtypes predominating in the central nervous system (Philipp *et al*., 2002).

α_2-Adrenoceptor agonists such as clonidine produce hypothermia in wild-type (WT) mice, and hypothermia involves α_{2A}-adrenoceptors because it is absent in α_{2A}-KO mice (Hunter *et al*., 1997; Zarrindast *et al*., 2003; Bexis and Docherty, 2005). The hypothermic response elicited by clonidine is thought to be primarily mediated by activation of post-synaptic α_{2A}-adrenoceptors in the pre-optic area of the hypothalamus (Myers *et al*., 1987). α_{2A}-Adrenoceptors are also involved in the MDMA-induced hyperthermia. In the presence of the α_{2A}-adrenoceptor antagonist BRL44408, the monophasic hyperthermic response produced by MDMA in WT mice became a biphasic response with an initial hypothermia followed by a small increase in body temperature (Bexis and Docherty, 2005) (see Figure 2). Similarly, when α_{2A}-KO mice were injected with MDMA, a biphasic response was seen, hypothermia followed by hyperthermia (Bexis and Docherty, 2005) (see Figure 2). The results are surprising because α_{2A}-adrenoceptors are involved in producing hypothermia.

As well as being found post-synaptically where they mediate hypothermia (Myers *et al*., 1987), α_2-adrenoceptors are also found pre-synaptically as inhibitory receptors regulating release of noradrenaline (autoreceptors) and other neurotransmitters, such as dopamine and 5-HT (heteroceptors), in the central and peripheral nervous systems (Philipp *et al*., 2002, Brede *et al*., 2003). Because the monoaminergic systems are interconnected and can influence each other, it could be suggested that under the conditions of increased extracellular levels of the three monoamines produced by MDMA, concomitant activation of the pre-synaptic α_{2A}-adrenoceptor results in a component of the hyperthermic response. In the absence of α_{2A}-adrenoceptors, this component of the hyperthermia is absent, and the resultant changes in levels of dopamine, 5-HT and possibly other neurotransmitters such as GABA, leads to the hypothermic component seen in α_{2A}-KO mice.

In addition to the predominant α_{2A}-adrenoceptor, it has been demonstrated that α_{2C}-adrenoceptors also function as a pre-synaptic regulator of noradrenaline release, both centrally and peripherally, but they are more prominent in sympathetic nerve endings than central adrenergic neurons (Ho *et al*., 1998; Philipp *et al*., 2002). The α_{2C}-adrenoceptors have also been shown to be involved in inducing a hypothermic response because in the absence or over-expression of α_{2C}-adrenoceptors, the hypothermic response to the α_2-adrenoceptor agonist, dexmedetomidine, is slightly decreased (17%) or increased (12%), respectively (Sallinen *et al*., 1997). Indeed, α_{2C}-adrenoceptor up-regulation has been shown to occur in α_{2A}-KO mice to partly replace α_{2A}-adrenoceptors prejunctionally in rat vas deferens (Ho *et al*., 1998; Hein *et al*., 1999; Cleary *et al*., 2002b). However, clonidine had no significant effect on temperature in α_{2A} knock-out mice (Bexis and Docherty, 2005), suggesting that either that the α_{2C}-adrenoceptor component is small, or that clondine has low potency at α_{2C}-adrenoceptors. MDMA has similar affinities/potencies at α_{2A}- and α_{2C}-adrenoceptors in ligand binding (Lavelle *et al*., 1999) and functional studies (Rajamani *et al*., 2001), but the relative low importance of α_{2C}-adrenoceptors in hypothermia and the lack of hypothermia to MDMA in WT animals still argue for a hyperthermic action of MDMA by α_{2A}-adrenoceptor activation in WT mice.

Although vasoconstriction is mediated predominantly by α_1-adrenoceptors, α_2-adrenoceptors, particularly α_{2A}-adrenoceptors, also contribute to systemic vasoconstriction (Docherty, 1998; Duka *et al*., 2000).). α_{2C}-Adrenoceptors are present on veins (Gavin *et al*., 1997) and on cutaneous arteries, and have been shown to be involved particularly in cold-induced vasoconstriction (Chotani *et al*., 2000).

In terms of prejunctional α_{2A}-adrenoceptor potency in rat vas deferens, the potency order agreed with the ligand binding affinity order of MDMA > MDA > MDEA (Bexis and Docherty, 2006).

MDMA also induces hyperthermia by central sympathetic activation of cutaneous vasoconstriction, reducing the ability to dissipate heat (Pedersen and Blessing, 2001).

Cardiac actions of MDMA and derivatives

Increased cardiac function can contribute to hyperthermia by increased blood pressure and flow, or be a result of hyperthermia. In humans, both MDA and MDEA increased heart rate (Gouzoulis *et al*., 1993; Hegadoren *et al*., 1999). In the anaesthetized rat, MDA elicited an initial bradycardia (McDaid and Docherty, 2001). All three agents tended to increase heart rate later, but this reached significance only for MDEA. These changes may be at least partly baroreflex responses to changes in blood pressure, given that MDA produced the largest increase in blood pressure and MDEA produced a fall. In conscious animals and humans, MDMA has been shown to produce no change in heart rate, bradycardia (at high doses) or tachycardia (O'Cain *et al*., 2000; Pedersen and Blessing, 2001; Badon *et al*., 2002; Cole and Sumnall, 2003a).

In the rat isolated right ventricle, MDMA potentiated contractions to noradrenaline, but not isoprenaline, suggesting an action at the noradrenaline transporter (Al-Sahli *et al*., 2001). These results suggest cardiac stimulant actions of MDMA, at least partly involving indirect sympathomimetic actions.

β_3-Adrenoceptors

There are probably a complex series of physiological changes involved in the production of the hyperthermic response that follows MDMA administration to the rat. Gordon *et al*. (1991) examined metabolic rate, evaporative water loss and rectal temperature following injection of the drug when rats were housed in ambient temperature of 10, 20 and 30°C. When compared to saline-injected control animals, it was found that MDMA increased both the metabolic rate and evaporative water loss. These changes involve both the hypothalamic–pituitary–thyroid axis (Sprague *et al*., 2003) and β_3-adrenoceptor activation in brown adipose tissue (Sprague *et al*., 2004), which induces heat generation through activation of uncoupling protein (Mills *et al*. 2004). The problem of hyperthermia, however, seems to be related to the fact that this heat generation is not followed by activation of heat loss mechanisms.

Studies in rat have demonstrated that in addition to α1-adrenoceptors, β_3-adrenoceptors may also be involved in MDMA-induced hyperthermia (Sprague *et al*., 2003, Sprague *et al*., 2004; 2005). In a series of studies, Sprague *et al*. have examined the involvement of β_3-adrenoceptors in the temperature actions of MDMA. In Sprague-Dawley rats, MDMA produced increases in rectal and skeletal muscle temperatures, but thyroidectomy revealed a hypothermic response to MDMA (Sprague *et al*., 2003). The β-antagonist cyanopindolol attenuated the increase in skeletal muscle temperature; the α_1-adrenoceptor antagonist prazosin attenuated both skeletal and rectal temperatures, but the combination of prazosin and cyanopindolol abolished the effects of MDMA on temperature (Sprague *et al*., 2003). The combination of α_1-adrenoceptor antagonism with prazosin and β_3-adrenoceptor antagonism with SR59230A, or the combined β_3- and α_1-adrenoceptor antagonist carvedilol (Qvigstad *et al*., 2005), abolished the rise in core temperature to MDMA (Sprague *et al*., 2004; 2005). These studies suggest major roles for α_1- and β_3-adrenoceptors in the temperature actions of MDMA.

Pretreatment of mice with SR59230A (5 mg·kg^{-1}), a concentration that has been demonstrated to prevent brown adipose tissue thermogenesis (Manara *et al*., 1996), altered the monophasic hyperthermic response produced by MDMA to a biphasic response, with an initial hypothermic response followed by a hyperthermic response (Bexis and Docherty, 2009). However, the hypothermic responses were similar in magnitude to the responses seen when mice were pretreated with prazosin (0.1 mg·kg^{-1}) (Bexis and Docherty, 2008). A problem in studies of responses mediated by β_3-adrenoceptors is the lack of selectivity of antagonists available. Although SR59230A has been commonly described as highly selective for β_3-adrenoceptors (pA2 of 8.76: Nisoli *et al*., 1996), it may not be selective for human β_3-adrenoceptors over other β-adrenoceptors (Vrydag and Michel, 2007). There is increasing evidence, both from functional and radioligand binding studies, to suggest that SR59230A also has antagonistic actions at α_1-adrenoceptors (pKi/pK_B values of 6.75–6.25: Brahmadevara *et al*., 2004; Leblais *et al*., 2004; Bexis and Docherty, 2009). Doses of SR59230A (5 mg·kg^{-1}) would be high enough to produce marked block of α_1-adrenoceptors. In the presence of a low concentration of SR59230A (0.5 mg·kg^{-1}), the temperature response produced by MDMA in the mouse remained monophasic, but the maximum temperature reached in the delayed hyperthermia was slightly, but significantly, reduced (Bexis and Docherty, 2009), so that there is a possible small β_3-adrenoceptor-mediated component to the hyperthermia to MDMA in the mouse (Bexis and Docherty, 2009) (see Figure 2). These results suggest a lesser role for β_3-adrenoceptors in the hyperthermia to MDMA in the mouse (Bexis and Docherty, 2009) than the rat (Sprague *et al*., 2004). Putative β_3-adrenoceptor antagonists should be investigated taking into consideration possible α_1-adrenoceptor antagonist actions.

Locomotor activity

In addition to hyperthermia, MDMA also induces a dose-dependent increase in locomotor activity in rats. It is therefore reasonable to examine whether the hyperthermia results from increased activity. All evidence to date argues against this supposition. The increased locomotor activity is unchanged when animals are placed in low ambient room temperature conditions, which prevents hyperthermia, and also in raised room temperature conditions, which enhances hyperthermia (Dafters, 1994; 1995; O'Shea *et al*. 2006). The recent study of Rodsiri *et al*. (2008) also failed to detect any relationship between the body temperature and the simultaneously measured locomotor response following administration of two different doses of MDMA in rats housed in normal ambient temperature.

Evidence suggests that MDMA-induced hypermotility involves activation of multiple 5-HT receptors and an interaction of dopamine and 5HT (Bankson and Cunningham, 2002; Cole and Sumnall, 2003a). In a study in rats, all three amphetamine derivatives tended to cause an increase in locomotor activity, but this reached significance only for MDA (Bexis and Docherty, 2006). There is also evidence that α_{2A}-adrenoceptors mediate inhibition of locomotion (Lähdesmäki *et al*., 2003), and the α_{2A}-adrenoceptor antagonist BRL 44408 revealed locomotor actions of MDMA (Bexis and Docherty, 2006).

Jaw clenching reported with the use of MDMA (Hayner and McKinney, 1986; McCann *et al*., 1996) may involve α_2-adrenoceptor-mediated inhibition of the jaw opening reflex (Arrue *et al*., 2004), presumably by a central action. Acute psychiatric complications of MDMA, including panic attacks (McCann *et al*., 1996), may also involve noradrenergic mechanisms and α_2-adrenoceptors.

Studies on temperature effects in monkeys and humans

Relatively few studies have been published on the effects of MDMA in either monkeys or humans. However, those that have suggest that the response of both monkeys and humans may differ in some important respects from that seen in rats.

MDMA can induce hyperthermia in both rhesus monkeys (Crean *et al*., 2006; 2007; Taffe *et al*., 2006; Banks *et al*., 2007; Von Huben *et al*., 2007) and humans, both when administered at modest doses (Liechti and Vollenweider, 2000a,b; Freedman *et al*., 2005), as well as at doses inducing an acute toxic response (e.g. Brown and Osterloh, 1987; Dowling *et al*., 1987; McCann *et al*., 1996; Farré *et al*., 2007).

However, in contrast to rats where one can induce hypothermia or hyperthermia depending on the ambient room temperature conditions in which the rats are housed (see earlier), MDMA administration to monkeys (Von Huben *et al*., 2007) and humans (Freedman *et al*., 2005) results in hyperthermia in low, normal or high ambient temperature conditions. There appears to be no association in monkeys between hyperthermia and increased locomotor activity (Taffe *et al*., 2006; Crean *et al*., 2006; 2007) which is consistent with data found in rats (see earlier). Liechti and Vollenweider (2000b) observed that neither citalopram nor haloperidol block MDMA-induced hyperthermia in humans and similar data have been reported in rats (Mechan *et al*., 2002; Piper *et al*., 2008). The 5-HT_{2A} antagonist ketanserin has, however, been

reported by Liechti *et al*. (2000) to attenuate MDMA-induced hyperthermia in normal volunteers.

Interestingly, it has recently been reported that core temperature can alter the pharmacokinetic parameters and metabolism of MDMA in both rats (Goni-Allo *et al*., 2008) and monkeys (Banks *et al*., 2007).

Conclusions

It was recently pointed out elsewhere (Green *et al*., 2009; de la Torre *et al*., 2009) that future studies on the physiological and psychological effects of MDMA must include much more awareness of the pharmacokinetics of MDMA in different species. Ideally, such studies should include much more information than simple exposure measurements, and include information on drug exposure, half-life, active metabolites (of which MDMA has several) and even plasma protein binding which can vary markedly between species [see Gabrielsson and Green (2009) for a full discussion of the use of quantitative pharmacology in experimental pharmacology]. The absence of such information in most studies on MDMA naturally inhibits our ability to draw many firm conclusions on the effects of MDMA on body temperature in different species. However, it is also clear from this review that the effect of MDMA on body temperature is complex because the drug has actions on all the major monoamine neurotransmitters (5-HT, dopamine and noradrenaline) both releasing the amines from nerve endings and also acting on their receptors. These neurotransmitters interact in complex ways both centrally and peripherally to control temperature with actions involving both central thermoregulation and peripheral changes in blood flow and brown adipose tissue thermogenesis. Given this information, it seems unlikely that any single pharmaceutical compound is going to be effective in treating acute MDMA-induced hyperthermia, and careful body cooling of affected persons remains, at present, the principal clinical approach. It has been suggested that atypical neuroleptics with 5-HT_{2A} and dopamine (and indeed α_1-adrenoceptor) antagonist actions such as risperidone (Shioda *et al*., 2008) and clozapine (Blessing *et al*., 2003; 2006) could prove of value in treating potentially fatal hyperthermia in humans. However, the fact that atypical neuroleptics can also induce the neuroleptic malignant syndrome (Farver, 2003) does mean that their use should be approached with caution.

Crucially, educating recreational users of the potential dangers of hyperthermia and control of the ambient temperature at clubs and other dance parties should remain key factors in fighting this potentially fatal problem.

Conflict of interest

The authors state no conflict of interest.

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