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RESEARCH PAPER

Role of α_1-adrenoceptor subtypes in the effects of methylenedioxy methamphetamine (MDMA) on body temperature in the mouse

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Background and purpose: We have investigated the ability of α_1-adrenoceptor antagonists to affect the hyperthermia produced by methylenedioxy methamphetamine (MDMA) in conscious mice.
Experimental approach: Mice were implanted with temperature probes under ether anaesthesia and allowed 2 weeks recovery. MDMA (20 mg kg^{-1}) was administered subcutaneously 30 min after vehicle or test antagonist or combination of antagonists and effects on body temperature monitored.
Key results: Following vehicle, MDMA produced a hyperthermia, reaching a maximum increase of 1.8 °C at 140 min. Prazosin (0.1 mg kg^{-1}) revealed an early significant hypothermia to MDMA of −1.94 °C. The α_{1A}-adrenoceptor antagonist RS 100329 (0.1 mg kg^{-1}), or the α_{1D}-adrenoceptor antagonist BMY 7378 (0.5 mg kg^{-1}) given alone, did not reveal a hypothermia to MDMA, but the combination of the two antagonists revealed a significant hypothermia to MDMA. The putative α_{1B}-adrenoceptor anatagonist cyclazosin (1 mg kg^{-1}) also revealed a significant hypothermia to MDMA, but actions of cyclazosin at the other α_1-adrenoceptor subtypes cannot be excluded.
Conclusions and implications: More than one subtype of α_1-adrenoceptor is involved in a component of the hyperthermic response to MDMA in mouse, probably both α_{1A}- and α_{1D}-adrenoceptors, and removal of this α_1-adrenoceptor-mediated component reveals an initial hypothermia.
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Keywords: MDMA; hyperthermia; α_1-adrenoceptor; α_{1A}-adrenoceptor; α_{1D}-adrenoceptor

Abbreviations: BMY 7378, (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4.5) decane-7,9-dione; MDMA, methylenedioxy methamphetamine; RS 100329, 5-methyl-3-(3-(4-(2-(2,2,2,-trifluoroethoxy)phenyl)-1-piperazinyl)propyl)-2,4-(^{1}H)-pyrimidinedione; WT, wild type

Introduction

Hyperthermia is a life-threatening acute consequence of methylenedioxy methamphetamine (MDMA) toxicity and is often seen when the drug is used at a rave, an environment where ambient temperature tends to be high and there is excessive physical exertion. In animal studies, it has also been shown that MDMA disrupts thermoregulation, causing either hypo- or hyperthermia depending on the ambient temperature (Malberg and Seiden, 1988). The mechanism(s) by which MDMA disrupts thermoregulation is still unclear, but both central and peripheral mechanisms have been implicated. As 5-hydroxytryptaminergic, noradrenergic and dopaminergic neurotransmitter systems have all been implicated in the mediation of hypothermia and hyperthermia, acute increases in these neurotransmitters induced by MDMA (White *et al.*, 1996), or agonist actions of MDMA at receptors for these neurotransmitters (Lavelle *et al.*, 1999; McDaid and Docherty 2001), may therefore influence the thermoregulatory system. Recently, we demonstrated that α_2-adrenoceptors are involved in MDMA-induced hyperthermia and, in the absence of α_{2A}-adrenoceptors, the monophasic hyperthermic response produced by MDMA became a biphasic response: hypothermia followed by hyperthermia (Bexis and Docherty, 2005). It is clear from our previous studies that α_2-adrenoceptors alone do not mediate all of the MDMA-induced hyperthermia (Bexis and Docherty, 2005). In addition to α_2-adrenoceptors, MDMA also has an affinity, although lower, for α_1-adrenoceptors and β-adrenoceptors in the brain (Battaglia *et al.*, 1988). Both α_1- and β_3-adrenoceptors have been shown to be involved in thermogenesis, and antagonism of α_1- and β_3-adrenoceptors reduces or abolishes MDMA-induced hyperthermia in the rat (Sprague *et al.*, 2003, 2004).

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It is established that there are three functional α_1-adrenoceptor subtypes: α_{1A}, α_{1B}, α_{1D} (see Guimaraes and Moura, 2001). The distribution of these α_1-adrenoceptor subtypes varies between organs and tissues, as does their functional response. The main aim of this study was to investigate the role of α_1-adrenoceptor subtypes in MDMA-mediated hyperthermia using the non-selective α_1-adrenoceptor antagonist prazosin ($\alpha_{1A,1B,1D}$) and the selective α_1-adrenoceptor subtype antagonists 5-methyl-3-(3-(4-(2-(2,2,2,-trifluoroethoxy)phenyl)-1-piperazinyl)propyl)-2,4-(^{1}H)-pyrimidinedione (RS 100329) (α_{1A}) (Williams *et al.*, 1999), BMY 7378 (α_{1D}) (Stone *et al.*, 2001) and cyclazosin (putative α_{1B}) (see Discussion) in wild-type (WT) mice.

Some of these results have been published in abstract form (Bexis and Docherty, 2006).

Methods

All studies conformed to the Declaration of Helsinki and were approved by the Department of Health and by the RCSI Research Ethics Committee. Male C-57 WT mice (22–35 g) were obtained from Harlan UK Limited (Shaw's Farm, Blackthorn, Bicester, UK).

Radiotelemetry

Under ether anaesthesia, animals were implanted with a radiotelemetric device enabling measurement of core body temperature (TAC50-PXT; Data Sciences International, St Paul, MN, USA). The implant was placed in the abdominal cavity and the abdomen was then closed. Animals were given temgesic (buprenorphine hydrochloride 0.05 mg kg^{-1}, Schering-Plough, Welwyn, UK) subcutaneously (s.c.) post-operatively and allowed to recover for 14 days before experiments were performed.

Animals were housed individually, and home cages and bedding were used during temperature monitoring. On experimental days, a PhysiolTel-Receiver (model RPC-1) was placed under each animal cage, enabling recording of core body temperature. Data signals were acquired 90 min prior to and 300 min after vehicle (1 ml kg^{-1}) or MDMA (20 mg kg^{-1}) administration. All recordings were obtained at room temperature (23 ± 0.2 °C).

Drug treatments

Since treatment of the animals and recordings were performed in the laboratory and not in the animal facility, animals were allowed to acclimatize, in their home cages, to the surroundings in the laboratory for 2 days (5–6 h per day) before administration of any drugs. Animals were injected s.c. with the non-selective α_1-adrenoceptor antagonist prazosin (0.1 mg kg^{-1}), selective α_{1A}-adrenoceptor antagonist RS 100329 (0.1 mg kg^{-1}), selective α_{1D}-adrenoceptor antagonist BMY 7378 (0.5 mg kg^{-1}), RS 100329 (0.1 mg kg^{-1}) plus BMY 7378 (0.5 mg kg^{-1}) or the putative α_{1B}-adrenoceptor antagonist cyclazosin (1.0 mg kg^{-1}). Antagonists were administered 30 min prior to the injection of vehicle (1 ml kg^{-1}) or MDMA (20 mg kg^{-1}).

Statistics

Values are mean ± s.e.mean from 5–9 experiments. Responses were compared between groups by repeated measures analysis of variance followed by the Bonferroni or Dunnett test. Statistical and graphical analysis was carried out using GraphPad Prism for Macintosh computers.

Drugs

(+/−)-3,4-methylenedioxy methamphetamine hydrochloride (MDMA) (Research Biochemicals, Natick, MA, USA and NIDA, Bethesda, MD, USA); BMY 7378 ((8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4.5) decane-7,9-dione); Tocris, Bristol, UK); cyclazosin HCl (Research Biochemicals International, Natick, MA, USA, now part of Sigma-Aldrich); prazosin HCl (Sigma, Dublin, Ireland); RS 100329 (5-methyl-3-(3-(4-(2-(2,2,2,-trifluoroethoxy)phenyl)-1-piperazinyl)propyl)-2,4-(^{1}H)-pyrimidinedione; Tocris, Bristol, UK). All drugs were dissolved in distilled water.

Results

Resting core body temperature

The resting body temperatures for all groups prior to drug treatment were not significantly different (see Figures 1a and 2a). However, body temperature transiently increased after the administration of vehicle (for antagonist) or antagonists (Figures 1 and 2). In all studies in which vehicle replaced MDMA, irrespective of which antagonist was used, there were no significant differences between groups in the response to vehicle, whether or not data were expressed as absolute temperature or change in temperature (Figures 1a and b).

Effect of MDMA on core body temperature

Although baseline temperature did not significantly differ between groups prior to vehicle/antagonist and MDMA, baseline temperatures ranged between 35.4 and 36.2 °C, whereas maximum hypothermia was only about 2.0 °C (Figure 2a); therefore, effects of drugs on response to MDMA are best seen in terms of change in temperature from baseline (Figure 2b). Following vehicle injection, the administration of MDMA (20 mg kg^{-1}) resulted in hyperthermia (Figure 2b). Significant hyperthermia was observed at 70 min post-MDMA with the maximal temperature occurring at 130 min, after which temperature gradually declined.

Effect of the non-selective α_1-adrenoceptor antagonist prazosin on MDMA-induced hyperthermia

Prazosin (0.1 mg kg^{-1}), followed by a vehicle injection, did not produce any change in core body temperature when compared to the vehicle group (Figure 1).

Following pretreatment of mice with prazosin (0.1 mg kg^{-1}), MDMA administration produced a significant decrease in core temperature, which was then followed by an increase in core temperature, reaching temperatures similar to those obtained in mice treated with MDMA alone (Figure 2b).

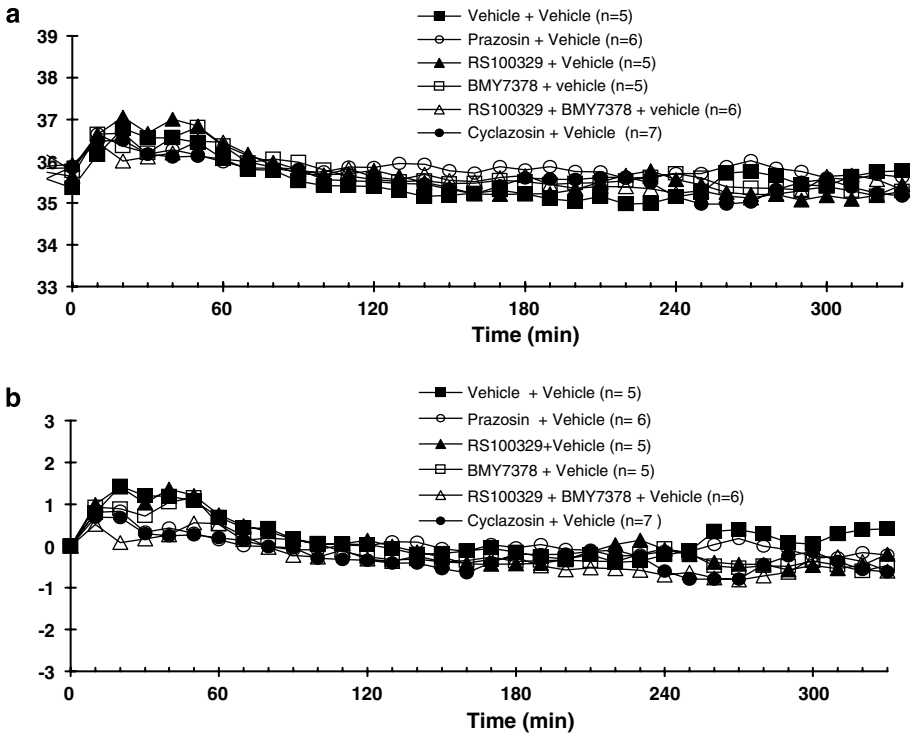

Figure 1 Core body temperature recordings in conscious WT mice given vehicle or antagonists 30 min prior to vehicle, at room temperature. All drugs or vehicles were administered s.c. Responses are expressed as (**a**) absolute body temperature and (**b**) change in body temperature. Vertical bars indicate the s.e.mean from 5–9 mice. Antagonist or vehicle was injected at time 0, and vehicle at 30 min.

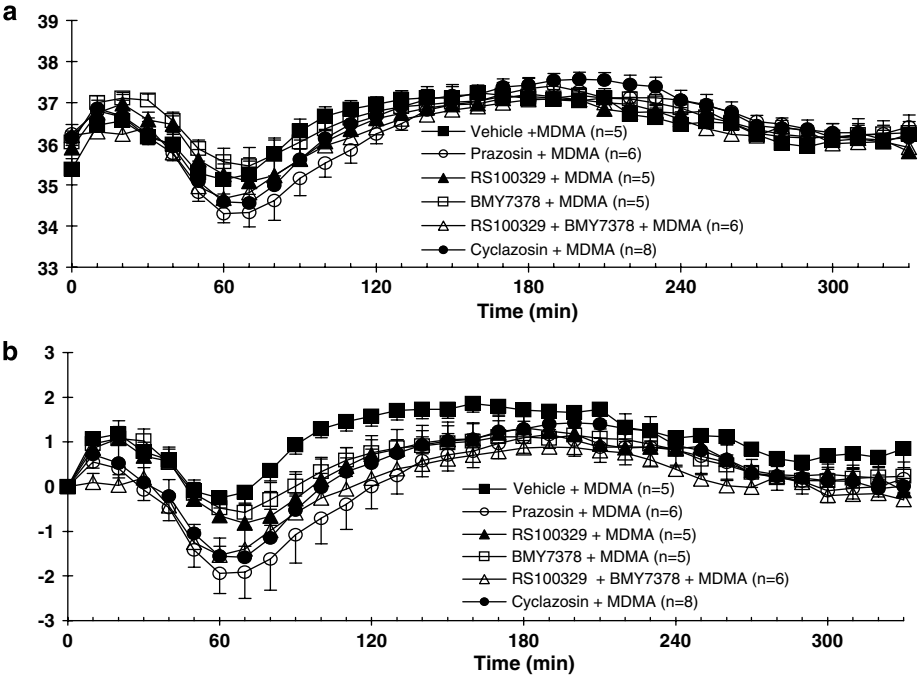

Figure 2 Core body temperature recordings in conscious WT mice given vehicle, prazosin (0.1 mg kg^{-1}), RS 100329 (0.1 mg kg^{-1}), BMY 7378 (0.5 mg kg^{-1}), RS 100329 (0.1 mg kg^{-1}) plus BMY 7378 (0.5 mg kg^{-1}) or cyclazosin (1 mg kg-^{1}) 30 min prior to administration of MDMA (20 mg kg^{-1}), at room temperature. All drugs were administered s.c. Responses are expressed as (**a**) absolute body temperature and (**b**) change in body temperature. Vertical bars indicate the s.e.mean from 5–9 mice. Antagonist or vehicle was injected at time 0, and MDMA at 30 min.

Effect of selective α_{1A}- and α_{1D}-adrenoceptor antagonists on MDMA-induced hyperthermia

The selective α_{1A}-adrenoceptor antagonist RS 100329 (0.1 mg kg^{-1}) or α_{1D}-adrenoceptor antagonist BMY 7378 (0.5 mg kg^{-1}) when given alone did not produce any change in core body temperature in relation to the vehicle group (Figure 1). Following pretreatment of mice with RS 100329 (0.1 mg kg^{-1}) or BMY 7378 (0.5 mg kg^{-1}), MDMA produced a

slight decrease in core body temperature that was not significantly different from temperatures obtained in mice treated with MDMA (post-vehicle) followed by a hyperthermic response (Figure 2b).

Co-administration of RS 100329 (0.1 mg kg^{-1}) and BMY 7378 (0.5 mg kg^{-1}) in vehicle experiments did not significantly change core body temperature compared to the vehicle group (Figure 1). Pretreatment of mice with RS 100329 (0.1 mg kg^{-1}) and BMY 7378 (0.5 mg kg^{-1}) in combination altered the monophasic hyperthermic response induced by MDMA to a biphasic response: a hypothermic response followed by a hyperthermic response (Figure 2b). The decrease in core temperature was significantly different from the effects of MDMA following vehicle and similar to the effect of MDMA post-prazosin (see above).

Effect of putative α_{1B}-adrenoceptor antagonist on MDMA-induced hyperthermia

The putative α_{1B}-adrenoceptor antagonist cyclazosin (1 mg kg^{-1}) in vehicle experiments did not significantly alter the body temperature when compared to vehicle group (Figure 1). The pretreatment of mice with cyclazosin significantly altered the effect of MDMA on core body temperature (Figure 2b). In the presence of cyclazosin, MDMA caused an initial decrease in core temperature that was significantly different from core temperatures in mice treated with MDMA post-vehicle. After reaching a minimum temperature 40 min after MDMA administration, core temperature began to rise, reaching temperatures similar to those observed in mice treated with MDMA post-vehicle (Figure 2b).

The onset of hypothermia in all of the treatment groups occurred 10 min after the injection of MDMA and a minimum core temperature was reached 40 min after drug administration. The maximum core temperature was reached between 120 and 160 min after the injection of MDMA, followed by a gradual decrease in core temperature towards baseline levels (Figures 2a and b). MDMA produced a significant hyperthermia ($P<0.05$) in all groups of experiment, irrespective of the antagonist employed, as compared to baseline absolute temperature (Figure 2b). Although all antagonist treatments tended to reduce the maximum hyperthermia to MDMA, only the combination of RS 100329 and BMY 7378 significantly reduced this ($P<0.05$; comparison of changes in body temperature from baseline) (see Figure 2b).

Discussion

Hyperthermia is one of the most detrimental acute toxic effects of MDMA ingestion in humans. The mechanism(s) by which MDMA may disrupt thermoregulation still remain elusive, hindering therapeutic intervention. It is well established that the sympathetic nervous system is involved in thermoregulation, both centrally and peripherally, in terms of both heat production and transfer. Changes in sympathetic outflow to cutaneous blood vessels will alter blood flow and thereby heat transfer, and the sympathetic innervation regulates brown adipose tissue metabolism, which is important for heat production (Morrison, 2004). Non-shivering thermogenesis may occur in skeletal muscle, and, in part, metabolic alterations occur as a result of changes to blood flow involving sympathetic nerves (Ye and Colquhoun, 1998). The hyperthermic response induced by MDMA has been suggested to be a result of both a reduction in the ability of the animal to dissipate heat and increased metabolism (Gordon *et al*., 1991; Pedersen and Blessing, 2001; Blessing *et al*., 2006). Indirect actions of MDMA to release noradrenaline (White *et al*., 1996) and direct agonist actions of MDMA at adrenergic receptors (Lavelle *et al*., 1999; McDaid and Docherty 2001) may be involved in the hyperthermic response.

Before discussing the results obtained, it is appropriate to consider the antagonist concentrations chosen. Prazosin (0.1 mg kg^{-1}, 0.24 μmol kg^{-1}) was chosen as a dose that revealed a significant hypothermia to MDMA, while producing only threshold effects at α_2-adrenoceptors (see below). BMY 7378 (0.5 mg kg^{-1}, 1.1 μmol kg^{-1}) was chosen to block α_{1D}-adrenoceptors (pKi of 8.78, -log M; Ki of 0.016 μM), with only threshold effects at α_{1A}-adrenoceptors (6.32, 0.48 μM) and limited effects at α_{1B}-adrenoceptors (6.74, 0.18 μM) (see Honner and Docherty, 1999). RS 100329 (0.1 mg kg^{-1}, 0.214 μmol kg^{-1}) was chosen to block α_{1A}-adrenoceptors (pKi of 9.6, 0.25 nM), with only limited effects at α_{1D}-adrenoceptors (7.9, 0.013 μM) and α_{1B}-adrenoceptors (pKi of 7.50, 0.032 μM) (Williams *et al*., 1999). Cyclazosin (1 mg kg^{-1}, 2.1 μmol kg^{-1}) was chosen to block α_{1B}-adrenoceptors, given the uncertainty of its potency at these receptors (pA_2/pKa values; 8.85, 1.4 nM, Marucci *et al*., 2005; 7.96, 0.011 μM, Stam *et al*., 1998).

In this study, we have demonstrated that specific blockade of α_1-adrenoceptors with prazosin altered the hyperthermic response to MDMA in mice. In the presence of prazosin, the monophasic hyperthermic response produced by MDMA in WT mice became a biphasic response, with an initial hypothermic response followed by a hyperthermic response. Our results are consistent with other studies demonstrating the involvement of α_1-adrenoceptors in the increase in core temperature seen after treatment with MDMA (Sprague *et al*, 2003). α_1-Adrenoceptors have been shown to be present on thermoregulatory pathways within the CNS, which regulate sympathetic outflow to cutaneous vessels and brown adipose tissue (Boulant 2000; Mallick *et al*., 2002). In the periphery, α_1-adrenoceptors are found on cutaneous blood vessels regulating blood flow by vasoconstriction. They are also found on brown adipocytes where their activation potentiates β-adrenoceptor-mediated thermogenesis (Zhao *et al*., 1997). MDMA-induced hyperthermia has been shown to involve both cutaneous vasoconstriction and brown adipose tissue thermogenesis due to central sympathetic activation (Pedersen and Blessing, 2001; Blessing *et al*., 2006). Therefore, it can be suggested that α_1-adrenoceptor blockade attenuates both central and peripheral sympathetic outflow to brown adipose tissue and cutaneous blood vessels, causing a decrease in heat production and an increase in heat loss leading to a decrease in body temperature. In addition to a decrease in sympathetic outflow, direct peripheral antagonism of α_1-adrenoceptors on the vasculature and brown

adipocytes could also contribute to the hypothermic response seen in animals pretreated with prazosin prior to the administration of MDMA.

As prazosin alone did not significantly affect temperature and as prazosin revealed a hypothermia to MDMA, it seems that prazosin targets α_1-adrenoceptors in vasculature and/or adipocytes that are tonically activated only in the presence of MDMA, or that block of α_1-adrenoceptors reveals a hypothermic response to MDMA involving other receptors.

Even when prazosin and other antagonists or combinations revealed a hypothermia to MDMA, a later hyperthermia developed. Although pharmacokinetic factors cannot be ruled out, it seems more likely that this can be explained by actions of MDMA on other receptor systems or another component of temperature regulation. It can be noted from Figure 2 that the recovery time from hypothermia to hyperthermia is similar for all drugs. Other authors have reported that prazosin can prevent but not reverse the hyperthermia to MDMA in mice (Fantegrossi *et al*., 2004), suggesting that the later hyperthermia following prazosin in our studies has little to do with pharmacokinetics and more to do with a later hyperthermic component to the response to MDMA.

Prazosin is an antagonist selective for α_1-adrenoceptors but displays high affinity for all the three subtypes: α_{1A}-, α_{1B}-, α_{1D}-adrenoceptors (pKi > 9) (Docherty, 1998; Guimaraes and Moura, 2001). All the three subtypes are found both centrally and peripherally, but their distribution and function varies between tissues. In the CNS, localization and the precise functional roles of the specific subtypes remain uncertain (Tanoue *et al*, 2002; Hague *et al*, 2003). In the vascular tree, all the three subtypes have been shown to be expressed and there is diversity in the α_1-adrenoceptor subtype mediating vascular contraction (Tanoue *et al*, 2002). Studies have shown that one or two of the α_1-adrenoceptor subtypes usually predominate in the contraction of a given blood vessel. Functional studies using isolated blood vessels suggest that α_{1A}- and α_{1D}-adrenoceptors primarily control vasoconstriction with a minor contribution from α_{1B}-adrenoceptors (Docherty, 1998; Guimaraes and Moura, 2001; Hague *et al*., 2003). Studies have also demonstrated that both α_{1A}- and α_{1D}-adrenoceptor mRNA expressions are high in brown adipose tissue, with α_{1B}-adrenoceptor expression being very low or not expressed at all (Granneman *et al*., 1997; Kikuchi-Utsumi *et al*, 1997; Chernogubova *et al*., 2005), although α_{1D}-adrenoceptor-binding sites were not detected (levels $<2\%$ of α_{1A} binding) (Granneman *et al*., 1997). However, in rat vas deferens, α_{1D}-adrenoceptors are difficult to detect by ligand-binding studies, except after chemical sympathectomy, because they are usually localized in junctional areas to mediate actions of nerve-released neurotransmitter (Cleary *et al*., 2004).

The main purpose of our study was to investigate the relative participation of the three α_1-adrenoceptor subtypes in the hyperthermic response induced by MDMA. We compared the effects of the α_{1A}-adrenoceptor selective antagonist RS 100329, the α_{1D}-adrenoceptor selective antagonist BMY 7378 and the putative α_{1B}-adrenoceptor antagonist cyclazosin on MDMA-induced hyperthermia. Individually, RS 100329 and BMY 7378 did not significantly alter the hyperthermic response produced by MDMA. However, when RS 100329 and BMY 7378 were administered simultaneously, a biphasic response to MDMA was obtained: hypothermia followed by hyperthermia. The hypothermic response was significantly different from the hyperthermic response seen in mice treated with MDMA alone. From these results, it is suggested that both α_{1A}- and α_{1D}-adrenoceptors are involved, and blockade of only one subtype was not sufficient to alter the thermoregulatory effect of MDMA. As BMY 7378, at a dose of 0.25 mg kg^{-1}, s.c., has been shown to reduce central 5-HT release (Hjorth *et al*, 1995), it cannot be ruled out that the reversal of the hyperthermic response also involves 5-HT$_{1A}$ autoreceptor agonism but admittedly only in the presence of α_{1A}-adrenoceptor blockade. Given the similar actions of prazosin, it is more likely that BMY 7378 is acting as an α_{1D}-adrenoceptor antagonist when effective in combination with RS 100329.

Admittedly, cyclazosin also revealed a biphasic response to MDMA. Discrepancies exist in the literature regarding the selectivity of cyclazosin. Studies have demonstrated that cyclazosin either is highly selective for α_{1B}-adrenoceptors (see Marucci *et al*, 2005) or shows relatively equal affinity for all three α_1-adrenoceptor subtypes (Stam *et al*, 1998). The hypothermic response seen with cyclazosin or with the co-administration of RS 100329 and BMY 7378 was similar to the hypothermic response seen with prazosin alone. Perhaps the crucial fact to suggest that α_{1B}-adrenoceptors are not important in these responses is that RS 100329 has limited potency, but it is more potent than BMY 7378, at α_{1B}-adrenoceptors (compare Williams *et al*., 1999 with values quoted in Honner and Docherty, 1999). Furthermore, RS 100329 has similar affinities for α_{1B}- and α_{1D}-adrenoceptors (Williams *et al*., 1999); therefore, any action at α_{1B}-adrenoceptors should be matched by action at α_{1D}-adrenoceptors. Hence, it is difficult to see how the combination of RS 100329 and BMY 7378 would have markedly more effect than RS 100329 alone at α_{1B}-adrenoceptors.

In a previous study, we demonstrated that α_{2A}-adrenoceptors were involved in the hyperthermic response produced by MDMA and in their absence a biphasic response was obtained (Bexis and Docherty, 2005). Briefly, α_2-adrenoceptors are found presynaptically as inhibitory receptors regulating the release of noradrenaline (autoreceptors) and other neurotransmitters, such as dopamine and 5-HT (heteroreceptors) in the central and peripheral nervous systems (Philipp *et al*., 2002; Brede *et al*., 2004), as well as being present postsynaptically in a number of cell types including smooth muscle. Since monoaminergic systems are interconnected and can influence each other, it was thereby suggested that, under the condition of increased extracellular levels of the three monoamines produced by MDMA, concomitant activation of neuronal α_{2A}-adrenoceptors could result in a component of the hyperthermic response. In addition, α_{2A}-adrenoceptors have been shown to contribute to systemic vasoconstriction (Gavin and Docherty, 1996; Duka *et al*, 2000) and thus may contribute to cutaneous vasoconstriction. However, α_{2A}-adrenoceptors have been demonstrated to counteract β-adrenoceptor-mediated lipolysis (Stich *et al*., 2003); therefore, it is perhaps surprising

that hypothermia results in the α_{2A}-adrenoceptor knock-out mouse.

As tests in α_{2A}-adrenoceptor knock-out mice or α_{2A}-adrenoceptor antagonism in WT mice also reveals a hypotensive component of the response to MDMA, we have to consider whether actions of prazosin or BMY 7378 in combination with RS 100329 could be explained by α_{2A}-adrenoceptor blockade. We have a number of reasons to suggest that the actions of these agents are mainly at α_1-adrenoceptors. Firstly, prazosin (0.1 mg kg^{-1}, 0.24 μmol kg^{-1}) would have threshold effects only at α_{2A}-adrenoceptors (pKi of 6.24, 0.58 μM) and minimal effects at α_{2B}-adrenoceptors (7.12, 0.08 μM) and α_{2C}-adrenoceptors (6.92, 0.12 μM) (Ho *et al*., 1998). Secondly, BMY 7378 (0.5 mg kg^{-1}, 1.1 μmol kg^{-1}) would have threshold effects at α_{2C}-adrenoceptors (pKi of 6.54, 0.29 μM), but no effect would be expected at α_{2A}-adrenoceptors (pKi of 5.48, 3.3 μM) or α2B-adrenoceptors (pKi of 5.23, 5.9 μM) (Cleary *et al*., 2005). Incidentally, BRL 44408 (1 mg kg^{-1}, 3.02 μmol kg^{-1}) would have major effects at α_{2A}-adrenoceptors (7.77, 0.017 μM) but threshold effects at α_{2B}-adrenoceptors (6.11, 0.78 μM) and α_{2C}-adrenoceptors (6.28, 0.52 μM) (Ho *et al*., 1998). Thirdly, RS 100329 (0.1 mg kg^{-1}, 0.214 μmol kg^{-1}) would have minimal effects at α_{2A}-adrenoceptors (prejunctional actions above 0.1 μM in functional studies; Cleary *et al*., 2003). Overall, it is difficult to see how α_2-adrenoceptor antagonism could explain actions of prazosin or BMY 7378/RS 100329 in this study. Indeed, BRL 44408 in the dose used is more likely to have a component of its actions at α_{1A}-adrenoceptors (pKi of 6.59, 0.26 μM; Cleary *et al*., 2003).

Our results further confirm the involvement of both α_{2A}- and α_1-adrenoceptors in MDMA-induced hyperthermia. Other studies have demonstrated the involvement of β_3-adrenoceptors in MDMA-induced hyperthermia, at least in rats (Sprague *et al*, 2004, 2005). However, antagonism of α-adrenoceptors does not prevent the later component of the hyperthermia produced by MDMA, suggesting that other neurotransmitters and/or hormones are involved. Changes in the level of thyroid hormone influence the thermogenic response of brown adipose tissue (Silva, 2003), and thyroid hormone is involved in MDMA-induced hyperthermia (Sprague *et al*., 2003). Centrally released 5-HT and dopamine have been demonstrated to increase thermoregulatory cutaneous and brown adipose tissue sympathetic outflow (Ootsuka *et al*, 2004; Ootsuka and Blessing, 2006). It has also been demonstrated that activation of the uncoupling protein-3, a skeletal thermogenic protein, is involved in MDMA-induced hyperthermia (Mills *et al*, 2003).

In conclusion, more than one subtype of α_1-adrenoceptor is involved in a component of the hyperthermic response to MDMA in mouse, probably both α_{1A}- and α_{1D}-adrenoceptors, and removal of this α_1-adrenoceptor-mediated component reveals an initial hypothermia.

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Conflict of interest

The authors state no conflict of interest.

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