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Role of α_{2A}-adrenoceptors in the effects of MDMA on body temperature in the mouse

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1 3,4-Methylenedioxymetamphetamine (MDMA) produces complex effects on body temperature, including hypo- and hyperthermic components that vary with ambient temperature and strain of rat. We have previously reported that MDMA is an α_2-adrenoceptor agonist, and α_2-adrenoceptor agonists such as clonidine produce hypothermia.

2 The purpose of this study was to investigate the effects of MDMA on core body temperature measured by radiotelemetry in conscious wild-type (WT) and α_{2A}-knockout (α_{2A}-KO) mice.

3 Clonidine (0.1 mg kg^{-1}, subcutaneously (s.c.)) produced a hypothermic response in WT mice, but did not significantly affect temperature in α_2-KO mice. MDMA (20 mg kg^{-1}, s.c.) produced a significant hyperthermia in WT mice beginning at approximately 100 min after injection, recovering by 300 min, but produced a biphasic response, hypothermia followed by hyperthermia, in α_2-KO mice.

4 In WT mice, following the α_{2A}-adrenoceptor antagonist 2-((4,5-dihydro-1H-imidazol-2-yl)methyl)-2,3-dihydro-1-methyl-1H-isoindole (1 mg kg^{-1}, s.c.), MDMA (20 mg kg^{-1}) produced an initial hypothermia.

5 Hence, α_2-adrenoceptor agonist actions of MDMA contribute to its effects on body temperature, but in a surprising way. Although selective α_{2A}-adrenoceptor agonism produces hypothermia, the α_{2A}-adrenoceptor actions of MDMA alter the body temperature response to MDMA from biphasic (hypothermia followed by hyperthermia) to monophasic hyperthemia.
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Abbreviations: BRL 44408, 2-((4,5-dihydro-1H-imidazol-2-yl)methyl)-2,3-dihydro-1-methyl-1H-isoindole; 5-HT, 5-hydroxytryptamine; KO, knockout; MDMA, 3,4-methylenedioxymethamphetamine; WT, wild type

Introduction

Hyperthermia is a life-threatening acute consequence of 3,4-methylenedioxymethamphetamine (MDMA) toxicity and is often seen when the drug is used at a rave, an environment where ambient temperatures tend 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 & Seiden, 1988). The mechanism by which MDMA disrupts thermoregulation is still unclear, but both central and peripheral mechanisms have been implicated. Since serotonergic, noradrenergic and dopaminergic neurotransmitter systems have all been implicated in the mediation of hypothermia and hyperthermia, acute increases in these neurotransmitters induced by amphetamine-like actions of MDMA (White *et al*., 1996), or agonist actions of MDMA at receptors for these neurotransmitters, may therefore influence the thermoregulatory system. It has also been shown that activation of the uncoupling protein-3 (UCP3), a skeletal thermogenic protein, may be involved in MDMA-induced hyperthermia (Mills *et al*., 2003). In addition to having an affinity for 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA) uptake sites through which it can increase neurotransmitter accessibility to pre- and postsynaptic receptors, MDMA also has an affinity for α_2-adrenoceptors in the brain (Battaglia *et al*., 1988). Direct agonist effects of MDMA on α_2-adrenoceptors have been demonstrated both *in vivo* and *in vitro* (Lavelle *et al*., 1999; McDaid & Docherty, 2001).

α_2-Adrenoceptors mediate pre- and postsynaptic actions of noradrenaline both in the central and peripheral nervous systems (Philipp *et al*., 2002). α_2-Adrenoceptors have been separated into three subtypes, α_{2A}-, α_{2B} and α_{2C} (Bylund *et al*., 1994), with α_{2A} and α_{2C} subtypes predominating in the central nervous system (Philipp *et al*., 2002). The term α_{2D}-adrenoceptor has previously been used for the species orthologue of the human α_{2A}-adrenoceptor, founds in rodents (see Docherty, 1998; Guimaraes & Moura, 2001). 2-((4,5-dihydro-1H-imidazol-2-yl)methyl)-2,3-dihydro-1-methyl-1H-isoindole (BRL 44408) is an antagonist with selectivity for α_{2A}-adrenoceptors (Young *et al*., 1989; see Docherty, 1998; Guimaraes & Moura, 2001).

Clonidine, an α_2-adrenoceptor agonist, induces a hypothermia by action at central α_{2A}-adrenoceptors (Zarrindast *et al*., 2003). It might be expected that actions of MDMA at α_2-adrenoceptors may alter the hypo-hyperthermic actions of the agent. Hence, the aim of this study was to investigate the role of α_{2A}-adrenoceptors in MDMA-mediated hyperthermia using both the selective α_{2A}-adrenoceptor antagonist BRL 44408 and wild-type (WT) and α_{2A}-adrenoceptor knockout (α_{2A}-KO) mice.

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Some of the results have been presented in abstract form (Bexis & Docherty, 2005).

Methods

Male WT and α_{2A}-KO mice (22–35 g) were obtained from Jackson Laboratories (Bar Harbor, ME, U.S.A.). All studies conform to the Declaration of Helsinki and have been approved by the Department of Health and by the RCSI Research Ethics Committee.

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, U.S.A.). 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, U.K., subcutaneously (s.c.)) postoperatively and allowed to recover for 14 days before experiments were performed.

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 from 90 min prior to and for 300 min after drug administration. All recordings were obtained at room temperature (23 $\pm$ 0.18°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. either with vehicle (1 ml kg^{-1}), MDMA (20 mg kg^{-1}) or clonidine (0.1 mg kg^{-1}). In interaction studies, the α_{2A}-adrenoceptor antagonist BRL 44408 (1 mg kg^{-1}) was given 30 min prior to the injection of vehicle or MDMA. Some animals were injected with test drug at least 2 days after injection of vehicle (1 ml kg^{-1}).

Drugs

MDMA (Research Biochemicals, Natick, U.S.A. and NIDA, Bethesda, U.S.A.); clonidine HCl (Tocris, Bristol, U.K.); BRL 44408 (Sigma, Dublin, Ireland).

Drugs were dissolved in distilled water.

Statistics

Values are mean $\pm$ s.e.m. from n experiments. Responses were compared between groups by repeated-measures analysis of variance (ANOVA) followed by the Bonferroni test. Statistical and graphical analysis was carried out using GraphPad Prism for Macintosh computers.

Results

Core body temperature

The resting body temperature was 35.8 $\pm$ 0.2°C in WT animals prior to vehicle; 36.6 $\pm$ 0.4°C in WT animals prior to BRL 44408 and 35.2 $\pm$ 0.3°C in α_{2A}-KO mice prior to vehicle, respectively (n = 5–6). The resting body temperature values of animals from the three groups were not significantly different. However, body temperature transiently increased after the administration of vehicle or drug (see Figure 1).

Administration of clonidine (0.1 mg kg^{-1}, s.c.) to WT mice resulted in hypothermia with a minimum core temperature being reached by 40 min, after which the temperature rose, gradually reaching baseline levels by 300 min (Figure 1). In contrast, clonidine had no significant effect on core body temperature in α_{2A}-KO mice (Figure 1).

The effects of MDMA on core body temperature in WT and α_{2A}-KO mice are shown in Figure 2. In WT mice, MDMA (20 mg kg^{-1}, s.c.) produced hyperthermia, reaching a maximum core temperature 130 min after administration of the

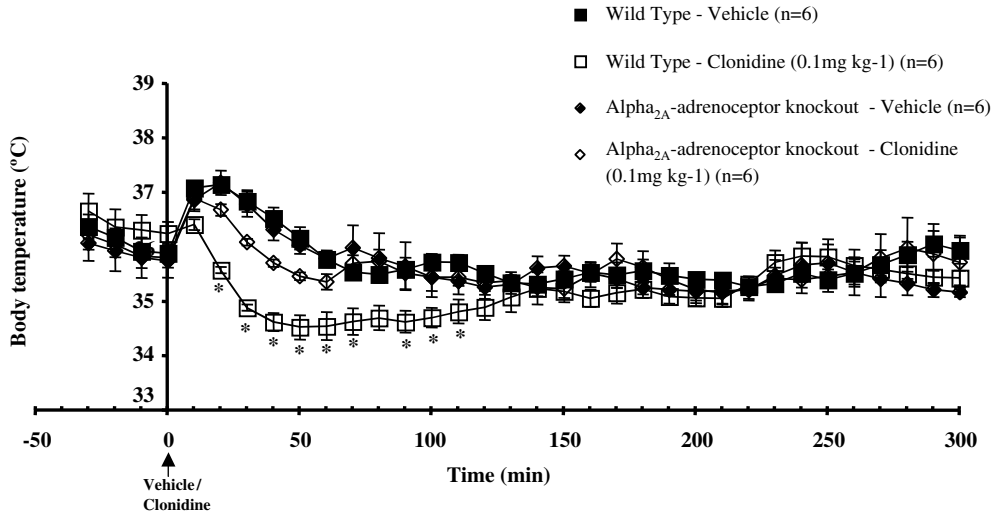

Figure 1 Core body temperature recordings in conscious WT or α_{2A}-adrenoceptor KO mice before and after vehicle or clonidine (0.1 mg kg^{-1}, s.c.) administration. Vertical bars indicate the s.e.m. from six mice. *P < 0.05 compared to the corresponding vehicle.

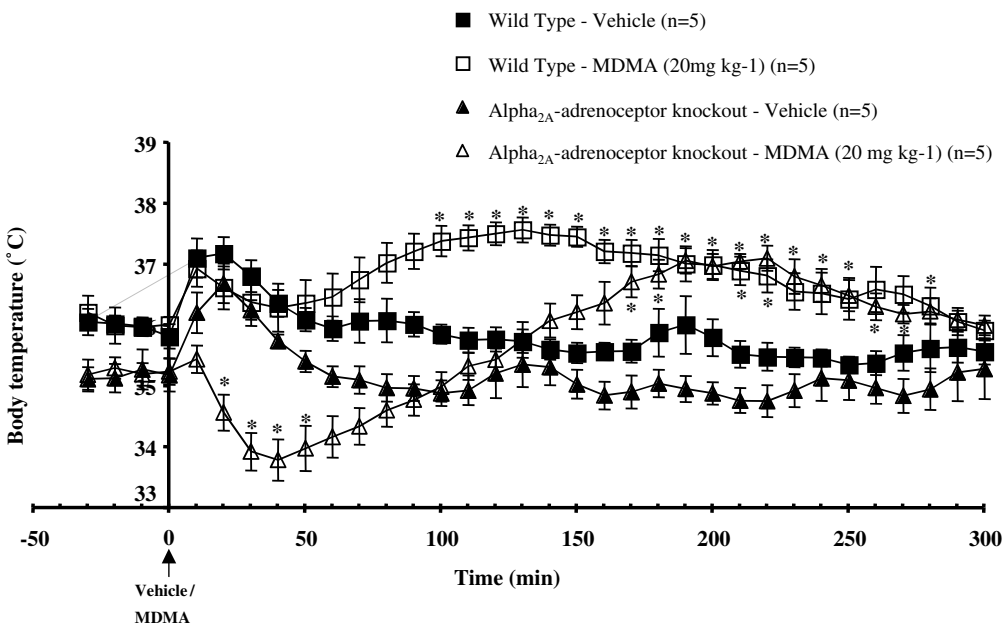

Figure 2 Core body temperature recordings in conscious WT or α_{2A}-adrenoceptor KO mice before and after vehicle or MDMA (20 mg kg^{-1}, s.c.) administration. Vertical bars indicate the s.e.m. from five mice. *$P<0.05$ compared to the corresponding vehicle.

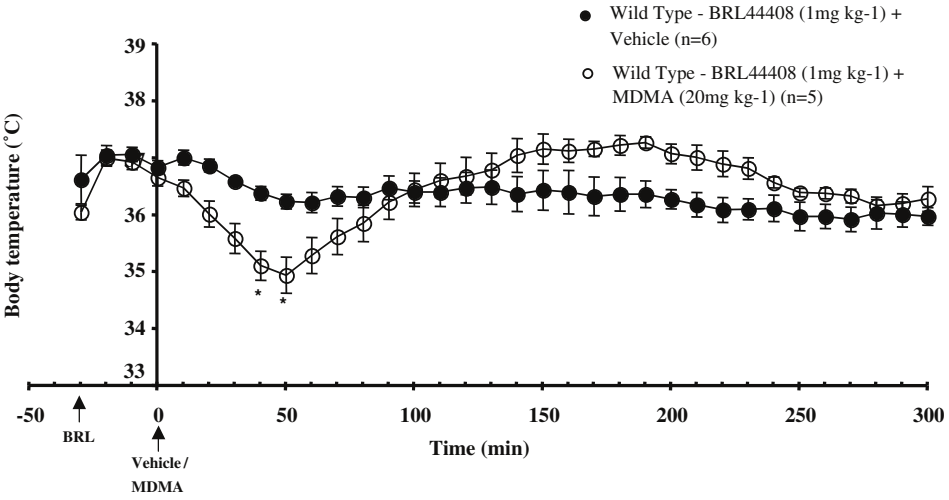

Figure 3 Core body temperature recordings in conscious WT mice administered BRL 44408 (1 mg kg^{-1}, s.c.) 30 min prior to vehicle or MDMA (20 mg kg^{-1}, s.c.). Vertical bars indicate the s.e.m. from five or six mice. *$P<0.05$ compared to the vehicle.

drug, followed by a gradual decrease in core temperature to baseline levels. In α_{2A}-KO mice, MDMA produced a biphasic response, consisting of an initial hypothermic response followed by hyperthermia (Figure 2). The onset of hypothermia in α_{2A}-KO mice occurred 20 min after the injection of MDMA and reached a minimum core temperature at 40 min after drug administration. The maximum core temperature was reached 220 min after the injection of MDMA, after which the temperature began to decline (Figure 2). In both WT and α_{2A}-KO mice, vehicle produced a transient increase in body temperature, followed by a relatively stable baseline (Figure 2).

In WT mice pretreated with BRL 44408 (1 mg kg^{-1}, s.c.), MDMA produced a significant decrease in core temperature, which was then followed by hyperthermia, although the hyperthermic response did not reach significance, as compared to the effects of vehicle (Figure 3).

Discussion

In this study, a combination of pharmacology and molecular genetics was used to determine the involvement of α_{2A}-adrenoceptors in the effects of MDMA on temperature. Before discussing the results obtained, we will first consider the drug doses chosen.

MDMA (1, 5 and 20 mg kg^{-1}) produces dose-dependent biphasic responses on blood pressure, pressor then depressor, in the anaesthetized rat and mouse (McDaid & Docherty,

2001; Vandeputte & Docherty, 2002), but only pressor responses in conscious rats (Bexis *et al*., 2003). In the mouse, Carvalho *et al*. (2002) found that MDMA (5, 10 and 20 mg kg^{-1}) produced dose-dependent increases in temperature, with the largest increase produced by MDMA (20 mg kg^{-1}). Hence, we chose a dose of MDMA (20 mg kg^{-1}) to assess the effects on temperature.

The dose of clonidine (0.1 mg kg^{-1}) was chosen based on the results of Zarrindast *et al*. (2003), who found that clonidine 0.05–0.1 mg kg^{-1} produced dose-dependent falls in temperature. We confirmed that s.c. administration of clonidine produced hypothermia in WT mice under our experimental conditions, and that clonidine did not significantly alter body temperature when administered to α_{2A}-KO mice, in agreement with Hunter *et al*. (1997). The hypothermic response elicited by clonidine is thought to be primarily mediated by activation of postsynaptic α_{2A}-adrenoceptors in the preoptic area of the hypothalamus (Myers *et al*., 1987). Admittedly, clonidine and MDMA have differing effects on blood pressure, as clonidine (0.1 mg kg^{-1}) lowers blood pressure by a central action (Zhu *et al*., 1999).

BRL 44408 shows selectivity for α_{2A} (formerly $\alpha_{2A/D}$) adrenoceptors over α_{2B} and α_{2C}. In our own ligand-binding studies, we have found that BRL 44408 shows 10–30-fold selectivity for α_{2A} (α_{2D}), with pK_i values ($-\log M$) of 7.77, 6.11 and 6.28 at α_{2A}, α_{2B} and α_{2C}-adrenoceptors, respectively (Cleary *et al*., 2002). However, BRL 44408 is also an antagonist at α_1-adrenoceptors, with a pK_i at α_{1A}-adrenoceptors of 6.59 (Cleary *et al*., 2003). Hence, BRL 44408 shows about 10-fold selectivity for α_{2A}- over other α_2-adrenoceptors and α_{1A}-adrenoceptors. In the pithed rat, we have shown that BRL 44408 (1 mg kg^{-1}) produces a 0.87 log unit shift in the postjunctional potency of the α_2-adrenoceptor agonist xylazine (Gavin & Docherty, 1996). Given that we could only expect 10-fold selectivity, 1 mg kg^{-1} was chosen as the dose to employ, producing approximately a 10-fold shift in xylazine (and presumably clonidine) potency *in vivo*. BRL 44408 (1 mg kg^{-1}) is likely to have only a small effect on blood pressure, but may prolong the pressor response to MDMA, as occurred in anaesthetized mouse by knockout of the α_{2A}-adrenoceptor (Vandeputte & Docherty, 2002).

In the present study, we have also demonstrated a role for α_{2A}-adrenoceptors in the MDMA-induced hyperthermia. In the presence of the α_{2A}-adrenoceptor antagonist BRL 44408, 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, although the hyperthermic response did not reach significance as compared to vehicle. Similarly, when α_{2A}-KO mice were injected with MDMA, a biphasic response was seen, hypothermia followed by hyperthermia. The results are surprising since α_{2A}-adrenoceptors are involved in producing hypothermia, as was also shown in the present study (see above). As well as being found postsynaptically where they mediate hypothermia (Myers *et al*., 1987), α_{2A}-adrenoceptors are also found presynaptically as inhibitory receptors regulating release of noradrenaline (autoreceptors) and other neurotransmitters, such as dopamine and 5HT (heteroceptors), in the central and peripheral nervous systems (Philipp *et al*., 2002; Brede *et al*., 2004). It has been demonstrated that the $5HT_2$ receptor antagonists, ketanserin and MDL100907, and fluoxetine, a 5-HT uptake inhibitor, attenuate the hyperthermic response mediated by MDMA in mice (Fantegrossi *et al*., 2003). In rats, the dopamine D_1-receptor antagonist SCH23390 produced a dose-dependent inhibition of hyperthermia induced by MDMA (Mechan *et al*., 2002). Hyperthermia elicited in mice by the 5HT-releasing amphetamine derivative, *p*-chloroamphetamine (PCA), is attenuated by ketanserin, SCH23390 and the dopamine depleter, α-methyl-*p*-tyrosine, but not by fluoxetine, or the 5HT depleter *p*-chlorophenylalanine (Sugimoto *et al*., 2000; 2001). This may indicate that dopamine plays a role in hyperthermia with $5HT_2$ receptors facilitating dopamine release or synthesis (Sugimoto *et al*., 2001). Since 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 presynaptic 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, 5HT and possibly other neurotransmitters lead 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 presynaptic regulator of noradrenaline, but they are more prominent in sympathetic nerve endings than central noradrenergic neurons (Ho *et al*., 1998; Philipp *et al*., 2002). The α_{2C}-adrenoceptors are also involved in inducing hypothermia, since, in the absence or overexpression 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 upregulation has been shown to occur to partly replace prejunctional α_{2A}-adrenoceptors in rat vas deferens (Hein & Kobilka, 1998; Cleary *et al*., 2002). However, clonidine had no significant effect on temperature in α_{2A}-KO mice (present results), suggesting that, either that the α_{2C}-adrenoceptor component is small (a small but insignificant hypothermic response is present in Figure 1), or that clondine has low affinity/efficacy 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 lack of 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. α_{2B}-adrenoceptors mediate a centrally mediated sympathoexcitatory response (Gavras *et al*., 2001), but it is not clear whether this response is important in the current study.

The effects of MDMA on body temperature in mice have been examined by other authors. After a single dose of MDMA (20 mg kg^{-1}), both a significant elevation and a significant drop in core temperature have been observed in mice (Miller & O'Callaghan, 1994; O'Shea *et al*., 2000; Johnson *et al*., 2002; Green *et al*., 2004). Although these discrepancies may be attributed partly to strain and sex differences, C57BL/6 mice were used in our study and all but one of the above studies (other study, Swiss Webster: O'Shea *et al*., 2000), and temperature changes were observed in both female and male mice (Miller & O'Callaghan, 1994; Johnson *et al*., 2002; Green *et al*., 2004).

Another mechanism by which MDMA induces hyperthermia is *via* cutaneous vasoconstriction, both direct and indirect

due to central sympathetic activation, reducing the animal's ability to dissipate heat (Pedersen & Blessing, 2001). Although vasoconstriction is mediated predominantly by α_1-adrenoceptors, α_2-adrenoceptors, particularly α_{2A}-adrenoceptors, also contribute to systemic vasoconstriction (Gavin & Docherty, 1996; Duka *et al*., 2000). In addition, 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). Therefore, in the absence of α_{2A}-adrenoceptors, a loss of a component of cutaneous vasoconstriction would not explain the hypothermic response unless cutaneous vasodilating actions of MDMA become evident in the absence of α_{2A}-adrenoceptors.

In conclusion, α_{2A}-adrenoceptor activation prevents MDMA from inducing an initial hypothermic response. α_{2A}-Adrenoceptor activation modulates other actions of MDMA, which in turn alter the temperature response from biphasic to monophasic.

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