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RESEARCH PAPER

Mechanisms mediating the ability of caffeine to influence MDMA ('Ecstasy')-induced hyperthermia in rats

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Background and purpose: Caffeine exacerbates the hyperthermia associated with an acute exposure to 3,4 methylenedioxymethamphetamine (MDMA, 'Ecstasy') in rats. The present study investigated the mechanisms mediating this interaction.
Experimental approach: Adult male Sprague-Dawley rats were treated with caffeine (10 $mg{\cdot}kg^{-1}$; i.p.) and MDMA (15 $mg{\cdot}kg^{-1}$; i.p.) alone and in combination. Core body temperatures were monitored before and after drug administration.
Key results: Central catecholamine depletion blocked MDMA-induced hyperthermia and its exacerbation by caffeine. Caffeine provoked a hyperthermic response when the catecholamine releaser D-amphetamine (1 $mg{\cdot}kg^{-1}$) was combined with the 5-HT releaser D-fenfluramine (5 $mg{\cdot}kg^{-1}$) or the non-selective dopamine receptor agonist apomorphine (1 $mg{\cdot}kg^{-1}$) was combined with the 5-HT_2 receptor agonist DOI (2 $mg{\cdot}kg^{-1}$) but not following either agents alone. Pretreatment with the dopamine D_1 receptor antagonist Schering (SCH) 23390 (1 $mg{\cdot}kg^{-1}$), the 5-HT_2 receptor antagonist ketanserin (5 $mg{\cdot}kg^{-1}$) or α_1-adrenoreceptor antagonist prazosin (0.2 $mg{\cdot}kg^{-1}$) blocked MDMA-induced hyperthermia and its exacerbation by caffeine. Co-administration of a combination of MDMA with the PDE-4 inhibitor rolipram (0.025 $mg{\cdot}kg^{-1}$) and the adenosine $A_{1/2}$ receptor antagonist 9-chloro-2-(2-furanyl)-[1,2,4]triazolo[1,5-C]quinazolin-5-amine 15943 (10 $mg{\cdot}kg^{-1}$) or the A_{2A} receptor antagonist SCH 58261 (2 $mg{\cdot}kg^{-1}$) but not the A_1 receptor antagonist DPCPX (10 $mg{\cdot}kg^{-1}$) exacerbated MDMA-induced hyperthermia.
Conclusions and implications: A mechanism comprising 5-HT and catecholamines is proposed to mediate MDMA-induced hyperthermia. A combination of adenosine A_{2A} receptor antagonism and PDE inhibition can account for the exacerbation of MDMA-induced hyperthermia by caffeine.
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Keywords: MDMA; caffeine; D-amphetamine; hyperthermia; dopamine; 5-HT serotonin; rat

Abbreviations: αMPT, alpha methyl para tyrosine; CYP 1A2, cytochrome P450 1A2; DOI, 2,5 dimethoxy-4-iodophenylaminopropane hydrochloride; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; HPLC, high performance liquid chromatography; MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethamphetamine; PCPA, para-chlorophenylalanine; PDE, phosphodiesterase

Introduction

Recently, we and others have reported that co-administration of caffeine exacerbates the acute toxicity of methylenedioxymethamphetamine (MDMA; Ecstasy) characterized by hyperthermia, tachycardia and death at higher doses (Camarasa *et al*., 2006; McNamara *et al*., 2006, 2007). This is a potentially serious drug interaction, the mechanism of which warrants further investigation. While it is appropriate to refer to hyperthermia and lethality independently, as the increase in body temperature may not be the sole contributing factor to mortality, hyperthermia is nevertheless a major feature of MDMA-induced toxicity, where body temperatures as high as 43°C have been reported in human users (Henry *et al*., 1992; Green *et al*., 2003). This can lead to other complications, such as rhabdomyolysis, disseminated intravascular coagulation and acute renal failure (Hegadoren *et al*., 1999). As a result, emphasis is placed on the body temperature response to

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MDMA to elucidate the mechanisms mediating the interaction.

There are several ways in which caffeine could interact with MDMA. As metabolism of both caffeine and the substituted amphetamines is catalysed by hepatic cytochrome enzymes and one of these is CYP1A2 (Carrillo and Benitez, 2000; Maurer *et al.*, 2000), it is possible that co-administration of caffeine could inhibit the metabolism of MDMA. Caffeine may also affect other processes including absorption, distribution or elimination processes. Importantly, MDMA has a non-linear pharmacokinetic profile, which has been attributed to a saturable or inhibitable metabolic pathway (De la Torre *et al.*, 2004; Farré *et al.*, 2004), making it especially vulnerable to drug–drug interactions. For this reason, the effect of co-administered caffeine on the availability of MDMA to the brain was determined.

It is widely reported that the pharmacological actions of MDMA result in the release of 5-HT and dopamine in several regions of the brain (see Green *et al.*, 2003; El-Mallakh and Abraham, 2007; Gudelsky and Yamamoto, 2008). Caffeine has also been reported to induce an increase in the release of 5-HT and dopamine in the cortex, hippocampus and striatum of freely behaving rats (Okada *et al.*, 1996; 1997; 1999; Acquas *et al.*, 2002). It is therefore conceivable that administration of caffeine could result in an augmentation of MDMA-induced 5-HT or dopamine release. It is also noteworthy that co-administration of caffeine with amphetamine or cocaine leads to a dramatic increase in seizures and mortality in rats in comparison with administration of amphetamine or cocaine alone (Derlet *et al.*, 1992). As amphetamine and cocaine increase extracellular dopamine levels, such interactions support a role for dopamine as an integral factor mediating severe adverse reactions associated with the concurrent use of caffeine.

There are biochemical mechanisms by which caffeine may interact with dopamine receptors or alter dopamine release. Specifically, caffeine is an adenosine receptor antagonist (Fredholm *et al.*, 1999; Nehlig, 1999), and this is a putative mechanism by which caffeine could mediate its effects on the toxicity of MDMA. In rats, caffeine induces hyperthermia, an increase in motor activity, blood pressure and heart rate. These effects of caffeine are mediated primarily through adenosine A_1 and A_2 receptors (receptor nomenclature follows Alexander *et al.*, 2009). Caffeine is also a weak inhibitor of phosphodiesterase (PDE) (Fredholm *et al.*, 1999), and therefore may augment intracellular cAMP/cGMP concentrations following MDMA administration, subsequent biogenic amine release and the activation of receptors which couple to adenylate/guanylate cyclase.

This study sets out to determine mechanisms that influence the ability of caffeine to exacerbate the hyperthermia associated with MDMA. The effects of MDMA alone or in combination with caffeine were examined in rats manipulated to influence central 5-HT and/or dopaminergic transmission in different ways. The roles of adenosine receptor antagonism and PDE inhibition were also assessed. The results show that both 5-HT and catecholaminergic mechanisms are relevant to the mechanism of this interaction. In addition, the ability of caffeine to exacerbate MDMA-induced hyperthermia relates to its action on adenosine A_{2A} receptors and PDE inhibition.

Methods

Animals

Animal care and all experimental protocols were in accordance with the guidelines of the Animal Welfare Committee, National University of Ireland, Galway and Trinity College Dublin, and was in compliance with the European Communities Council directive, 1986. Male Sprague-Dawley rats (200–250 g) were obtained from Harlan Olac, Bicester, UK. Animals were housed in groups of four in standard hard bottom polypropylene cages ($45 \times 28 \times 20$ cm) and with stainless steel lids, containing wood shavings. Animals had *ad libitum* access to food and water and were maintained at a constant temperature (20 ± 2°C) and at standard lighting conditions (12:12 h light–dark, lights on from 0800 to 2000 h). All animals were allowed 2 weeks acclimatization to the animal facility prior to any drug testing.

Recording of core body temperature

Core body temperatures were taken by inserting a digital rectal thermometer (Omron digital thermometer, MC-63B, Omron Health Care UK Ltd., Milton Keynes, UK) 3 cm into the rectum. Rats were lightly restrained by hand during the procedure, with a steady read-out of temperature obtained approximately 30 s after insertion of the probe. For each drug challenge, temperature was taken 1 h and immediately prior to drug administration, every 30 min for up to 2 h, and every hour up to 5 h post-challenge.

5-HT and catecholamine depletions

Central 5-HT depletion was induced by administration of the tryptophan hydroxylase inhibitor, para-chlorophenylalanine (PCPA; 150 mg·kg^{-1}; i.p.) once daily for 3 days. A 24-h period was allowed to elapse following the last treatment with PCPA prior to challenge with caffeine and MDMA. Catecholamine depletion was induced by administration of reserpine (5 mg·kg^{-1}, i.p.), which acts to deplete vesicular depots of catecholamines. 24 h later; this was followed with administration of the tyrosine hydroxylase inhibitor, α–methyl para tyrosine (αMPT; 150 mg·kg^{-1}, i.p.) twice, with doses 4 h apart. Drug challenge took place the following day. Combined treatment with reserpine and αMPT was warranted as neither alone is sufficient to induce a rapid and sustained decrease in catecholamine concentrations. These agents have been widely reported to selectively deplete the neurotransmitter system of relevance (see Linnet *et al.*, 1995; Ramos *et al.*, 2002; Yuan *et al.*, 2002; Harkin *et al.*, 2003).

Brain tissue preparation

Rats were killed by stunning followed by decapitation immediately following the last temperature measurement, and brains were dissected as previously described (Harkin *et al.*, 2001). The frontal cortex and hypothalamus were removed to determine the concentrations of 5-HT, dopamine and noradrenaline.

Determination of MDMA and methylenedioxyamphetamine (MDA) concentrations

Concentrations of MDMA and its metabolite methylenedioxyamphetamine (MDA) were measured in whole brains using high-performance liquid chromatography (HPLC), coupled with fluorescence detection as previously described (Brunnenberg *et al.*, 1998). MDMA and MDA were extracted from the tissue with 7.5% (v/v) perchloric acid (BDH Chemicals, Poole, UK) and 30 mM EDTA (BDH Chemicals). Forty microliters of the internal standard [N-methyl-dopamine (Sigma Aldrich, Dublin, Ireland) at a concentration of 250 ng·40 μL^{-1}] was added to extracted samples. Twenty microliters of samples and standards were injected onto a reverse column (Lichrosorb RP-18, 250 × 4 mm internal diameter, particle size 5 μm, Phenomenex, Macclesfield, Cheshire, UK). The mobile phase consisted of a 20 mM potassium dihydrogen phosphate : acetonitrile buffer 85:15 (v/v), adjusted to pH 3 with 8.5% orthophosphoric acid and was delivered at a flow rate of 1 mL per minute. Detection of MDMA in the column effluent was carried out using a fluorescence detector (Excitation λ 286 nm and Emission λ 322 nm). Quantification of MDMA and MDA was accomplished by measuring the area under the curve and comparing these values to that of a known external standard and the internal standard, using the computerized Class-VP chromatography laboratory software system (Shimadzu Europa GMBH, Duisburg, Germany). Results are expressed as ng MDMA or MDA per gram fresh weight of tissue.

Determination of 5-HT, dopamine and noradrenaline concentrations

Regional brain 5-HT and catecholamine (dopamine and noradrenaline) concentrations were also quantified using HPLC coupled to electrochemical detection as previously described (Harkin *et al.*, 2003). Mobile phase contained 0.1 M citric acid, 0.1 M sodium dihydrogen phosphate, 0.1 mM EDTA (BDH Chemicals), 1.4 mM octane-1-sulphonic acid (Sigma Aldrich) and 10 % (v/v) methanol (Laboratory-Scan, Ireland), and was adjusted to pH 2.8 using 4 N sodium hydroxide (BDH Chemicals). Standard amines (dopamine, noradrenaline, 5-HT, *N*-methyl-5-HT) were prepared separately in 10 mL HPLC mobile phase each, to give solutions of 1 mg·mL^{-1}, and these were then used to prepare 10 mL of standard mixture containing 2 ng·20 μL^{-1} of each compound in HPLC mobile phase. Brain tissue was sonicated in 1 mL of mobile phase, with 2 ng·50 μL^{-1} of N-methyl-5-HT (Sigma Aldrich) as internal standard. Homogenates were centrifuged at 15 000× *g* for 15 min, and a 20 μL sample of the resultant supernatant was injected onto a reverse phase column (Lichrosorb RP-18, 25 cm × 4 mm internal diameter, particle size 5 μm) for separation of the neurotransmitters (flow rate 1 mL per minute). Concentrations of dopamine, noradrenaline and 5-HT were quantified by electrochemical detection (Shimadzu), and chromatograms were generated using a Merck-Hitachi D-2000 integrator (Merck KGaA, Darmstadt, Germany). Results are expressed as ng of dopamine, noradrenaline and 5-HT per g fresh weight of tissue.

Experimental design

Study 1: Can central 5-HT or catecholamine depletion influence the ability of caffeine to exacerbate MDMA-induced hyperthermia? Control rats received a single administration of caffeine (10 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. Twenty-four hours following the last treatment with PCPA or αMPT, rats received a single administration of caffeine (10 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. Core body temperatures were recorded 1 h and immediately prior to and 30 min, 1, 1.5, 2, 3 and 5 h following drug administration, and cortical and hypothalamic tissue was obtained immediately following the last temperature measurement for the determination of 5-HT, dopamine and noradrenaline concentrations.

Study 2: Can caffeine influence the metabolism of MDMA? Rats received caffeine (10 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. Animals were killed 30 min, 1, 2, 4, 8 and 24 h following drug administration. Brain tissue was prepared for determination of MDMA and MDA concentrations as described earlier.

Study 3: Can caffeine influence the thermoregulatory response to D-fenfluramine and D-amphetamine alone or in combination? We further investigated if the ability of caffeine to exacerbate MDMA-induced hyperthermia could generalize to D-fenfluramine, a synthetic amphetamine that selectively induces the release of central 5-HT. Rats received a single administration of caffeine (10 mg·kg^{-1}, i.p.) and D-fenfluramine (5 mg·kg^{-1}, i.p.) alone and in combination. The dose of D-fenfluramine was selected from the descending limb of the dose-related core body temperature response in rats (Cryan *et al.*, 2000). To determine if the synergistic effects of caffeine with MDMA could generalize to D-amphetamine, which has predominant actions on central catecholamine systems, rats received a single administration of caffeine (10 mg·kg^{-1}, i.p.) and D-amphetamine (5 or 15 mg·kg^{-1}, i.p.) alone and in combination. As caffeine failed to influence the hyperthermic response to D-amphetamine alone, we examined the effect of co-administration of caffeine with D-fenfluramine (5 mg·kg^{-1}, i.p.) in combination with D-amphetamine (1 mg·kg^{-1}, i.p.). Animals were observed continuously following drug administration. Core body temperatures were obtained as previously described.

Study 4: Can caffeine influence the thermoregulatory response to 2,5 dimethoxy-4-iodophenyl-aminopropane hydrochloride (DOI) and apomorphine alone or in combination? We investigated if the ability of caffeine to exacerbate MDMA-induced hyperthermia might be simulated by co-administration with the non-selective 5-HT_2 receptor agonist DOI. Rats received a single administration of caffeine (10 mg·kg^{-1}, i.p.) and DOI (2 mg·kg^{-1}, i.p.) alone and in combination. The dose of DOI was selected from previous reports of 5-HT_2 receptor-mediated effects on core body temperature in rats (Mazzola-Pomietto *et al.*, 1995).

As caffeine failed to influence the hypothermic response to DOI, we determined if caffeine might influence the thermoregulatory response to the non-selective dopamine receptor agonist, apomorphine. Rats received a single administration of caffeine (10 mg·kg^{-1}, i.p.) and apomorphine (1 mg·kg^{-1}, i.p.) alone and in combination. The dose of apomorphine was selected from the descending limb of the dose-related core body temperature response in rats (Harkin *et al.*, 2000). As caffeine failed to influence the hypothermic response to apomorphine alone, we examined the effect of co-administration of caffeine with DOI (2 mg·kg^{-1}, i.p.) in combination with apomorphine (1 mg·kg^{-1}, i.p.). Core body temperatures were obtained as previously described.

Study 5: Effect of pretreatment with Schering (SCH) 23390 on the ability of caffeine to exacerbate MDMA-induced hyperthermia. R-ats were pretreated with a single administration of SCH 23390 (1 mg·kg^{-1}, i.p.), a selective dopamine $D_{1/5}$ receptor antagonist, and 30 min after, animals received a single administration of caffeine (10 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. SCH 23390 has previously been shown to attenuate MDMA-induced hyperthermia in rats (Mechan *et al.*, 2002). Core body temperatures were recorded as previously described.

Study 6: Effect of pretreatment with ketanserin, ritanserin and prazosin on the ability of caffeine to exacerbate MDMA-induced hyperthermia. Rats were pretreated with the 5-HT_2 receptor antagonists ketanserin (5 mg·kg^{-1}, i.p.) or ritanserin (1 mg·kg^{-1}, i.p.) and 30 min after, animals received a single administration of caffeine (10 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. The time interval and dose of ketanserin were selected from previous studies, where we have reported that ketanserin (5 mg·kg^{-1}) attenuates D-fenfluramine-induced hypothermia in rats (Cryan et al., 2000). Ritanserin was used at a dose of 1 mg kg^{-1} that is estimated to be 20 times the ED_{50} for inhibiting 5-HT_2-mediated behaviours (Goodwin and Green, 1985), and has previously been shown to reduce the benzodiazepine withdrawal syndrome in rats (Begg *et al.*, 2005). As ketanserin is known to block α_1 adrenoceptors (McCall and Schuette, 1984), rats were also pretreated with the α_1 adrenoceptor antagonist prazosin (0.2 mg·kg^{-1}, i.p.), and 30 min after, animals received caffeine (10 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. A previous report has shown that prazosin (0.2 mg·kg^{-1}) attenuates MDMA-induced hyperthermia in rats (Sprague et al., 2003).

Study 7: Can the adenosine receptor antagonists 9-chloro-2-(2-furanyl)-[1,2,4]triazolo[1,5-C]quinazolin-5-amine (CGS) 15943, DPCPX or SCH 58261 exacerbate MDMA-induced hyperthermia? We further investigated if the ability of caffeine to exacerbate MDMA-induced hyperthermia might be simulated by co-administration with the non-xanthine adenosine receptor antagonist CGS 15943. Rats received a single administration of CGS 15943 (10 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. Core body temperatures were obtained as previously described. As CGS 15943 failed to influence MDMA-induced hyperthermia, we next determined if co-treatment with the selective adenosine A_1 or A_{2A} receptor antagonists might influence the thermoregulatory response to MDMA. Rats received a single administration of the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 10 mg·kg^{-1}, i.p.) or the selective adenosine A_{2A} receptor antagonist SCH 58261 (2 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. The doses of CGS 15943 and DPCPX employed were chosen based on several previous experiments in which their central effects were investigated and demonstrated in rodents. Both antagonists possess a greater potency than caffeine as adenosine receptor antagonists (Jacobson *et al.*, 1996; Marston *et al.*, 1998; Ongini *et al.*, 1999). For the purpose of this study, we investigated a role for adenosine A_1 and A_{2A} receptors only as caffeine mainly binds adenosine A_1 and A_{2A} receptors with high affinity compared to adenosine A_{2B} or A_3 receptors (Fisone *et al.*, 2004).

Study 8: Can the PDE inhibitors pentoxyfylline, rolipram or zaprinast exacerbate MDMA-induced hyperthermia? As adenosine receptor antagonists failed to influence MDMA-induced hyperthermia, we next tested if the ability of caffeine to exacerbate MDMA-induced hyperthermia might be simulated by co-administration with the non-selective xanthine-based inhibitor of PDE, pentoxyfylline. Pentoxyfylline has a lower potency than caffeine as a PDE inhibitor (see Meskini *et al.*, 1994; Kruuse *et al.*, 2000). Rats received a single administration of pentoxyfylline (50 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination. Core body temperatures were obtained as previously described. As pentoxyfylline failed to influence MDMA-induced hyperthermia, we next determined if co-treatment with rolipram, a potent and selective inhibitor of the PDE-4 isoform, abundant in the brain, might influence the thermoregulatory response to MDMA. Rolipram is structurally unrelated to the methylxanthines, and is metabolized by a different pathway from that of caffeine (Müller *et al.*, 1996; Bian *et al.*, 2004). Finally, co-administration of the archetypical PDE-5 inhibitor zaprinast allowed investigation of the functional role of PDE-5 (for a review of PDE inhibitors, see Lugnier, 2006) in the interaction. Rats received a single administration of rolipram (0.5 mg·kg^{-1}, i.p.) or zaprinast (1 mg·kg^{-1}, i.p.) and MDMA (15 mg·kg^{-1}, i.p.) alone and in combination.

Study 9: Can co-treatment with a combination of CGS 15943, DPCPX or SCH 58261 and rolipram influence MDMA-induced hyperthermia? As pentoxyphyline, rolipram and zaprinast alone failed to influence the hyperthermic response to MDMA, we finally examined the effect of co-administration of rolipram (0.025 mg·kg^{-1}, i.p.) with each of the adenosine receptor antagonists CGS 15943 (10 mg·kg^{-1}, i.p.), DPCPX (10 mg·kg^{-1}, i.p.) or SCH 58261 (2 mg·kg^{-1}, i.p.) in combination with MDMA (15 mg·kg^{-1}, i.p.). Core body temperatures were obtained as previously described.

A summary of drug challenges undertaken to determine the mechanisms mediating the ability of caffeine to influence MDMA-induced hyperthermia is provided in Table 1.

Statistical analysis
All data were analysed by a three-factor repeated measures analysis of variance (ANOVA). If statistically significant changes were found, data were further analyzed using a Student Newman–Keuls *post hoc* comparison test. All data were deemed significant at $P < 0.01$.

Table 1 Summary of drug challenges undertaken to determine the mechanisms mediating the ability of caffeine to influence MDMA-induced hyperthermia

Target and rationale	*Drugs*
Role of central 5-HT or catecholamines in mediating the ability of caffeine to exacerbate the hyperthermic response to MDMA	p-CPA; Reserpine in combination with αMPT
Role of central 5-HT or catecholamines in simulating the ability of caffeine to exacerbate the hyperthermic response to MDMA	5-HT releaser, D-fenfluramine: Catecholamine releaser, D-amphetamine D-fenfluramine and D-amphetamine in combination
Role of 5-HT or dopamine receptor activation in simulating the ability of caffeine to exacerbate the hyperthermic response to MDMA	$5\text{-}HT_2$ receptor agonist, DOI Dopamine receptor agonist, apomorphine Alone or in combination
Role of dopamine D_1 receptor blockade on the ability of caffeine to exacerbate MDMA-induced hyperthermia	Dopamine D_1 receptor antagonist, SCH-23390
Role of $5\text{-}HT_2$ and α_1 adrenoceptor blockade on the ability of caffeine to exacerbate MDMA-induced hyperthermia	$5\text{-}HT_{2A}$ & α_1 adrenoceptor antagonist, ketanserin $5\text{-}HT_2$ receptor antagonist, ritanserin α_1 adrenoceptor antagonist. prazosin
Can adenosine receptor blockade exacerbate MDMA-induced hyperthermia?	$A_{1/2}$ adenosine receptor antagonist CGS 15943 A_1 adenosine receptor antagonist. DPCPX or A_{2A} adenosine receptor antagonist SCH 58261
Can PDE inhibition exacerbate MDMA-induced hyperthermia?	Non-selective PDE inhbitor, pentoxyfylline PDE 4 inhibitor, rolipram or PDE 5 inhibitor, zaprinast
Can combined adenosine receptor blockade and PDE inhibition influence MDMA-induced hyperthermia?	Rolipram in combination with CGS 15943, DPCPX or SCH 58261

Further details of drug challenges including dose, route and times are provided in the methods section.
CGS, 9-chloro-2-(2-furanyl)-[1,2,4]triazolo[1,5-C]quinazolin-5-amine; DOI, 2,5 dimethoxy-4-iodophenyl-aminopropane hydrochloride; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; MDMA, methylenedioxymethamphetamine; PDE, phosphodiesterase; SCH, Schering.

Materials
(+/–) MDMA HCl [The National Institute for Drug Abuse (NIDA) National Institutes of Health, Bethesda, MD, USA], caffeine, D-fenfluramine, D-amphetamine, SCH-23390, PCPA (para-chlorophenylalanine), αMPT (α-methyl-para-tyrosine), DOI (2,5-dimethoxy-4-iodophenyl-aminopropane-hydrochloride), ketanserin, DPCPX (8-cyclopentyl-1,3-dipropylxanthine), pentoxyfylline and zaprinast (Sigma Aldrich Ireland) were dissolved in 0.89% NaCl. Reserpine was dissolved in a few drops of 1% glacial acetic acid, and the volume made up with 0.89% NaCl. The final solution was pH adjusted to 7 with 0.1N NaOH. CGS-15943 (9-chloro-2-(2-furanyl)-[1,2,4]triazolo[1,5-C]quinazolin-5-amine) and rolipram (Sigma Aldrich Ireland) were prepared as micro-suspensions in 0.5% Tween saline. SCH 58261 (5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo [1,5-c] pyrimidine) (Sigma Aldrich Ireland) was prepared in 0.1% dimethyl sulfoxide in saline. All drugs were administered intraperitoneally (i.p.) in an injection volume of $1\ mL{\cdot}kg^{-1}$. Control rats received injections of vehicle alone in the same volume as the test groups.

Results

Effect of caffeine on MDMA-induced hyperthermia
ANOVA of body temperature showed effects of MDMA, [$F(1,20) = 73.09$, $P < 0.001$, caffeine [$F(1,20) = 7.47$, $P = 0.018$], time [$F(8160) = 63.54$, $P < 0.001$], MDMA × time [$F(8160) = 19.81$, $P < 0.001$] and caffeine × time [$F(8, 160) = 2.99$, $P < 0.01$]. *Post hoc* comparisons revealed that MDMA increased body temperature 0.5, 1, 1.5 and 2 h following drug administration when compared with vehicle-treated controls. Caffeine alone did not alter body temperature when compared with vehicle-treated controls. Caffeine increased MDMA-induced hyperthermia 30 min, 1, 1.5, 2, 3 and 4 h following drug administration compared with the MDMA alone treated group (Figure 1A).

5-HT depletion does not influence MDMA-induced hyperthermia or its exacerbation by caffeine
PCPA-induced 92 and 88% depletions in cortical (36 ± $5\ ng{\cdot}g^{-1}$) and hypothalamic (120 ± $21\ ng{\cdot}g^{-1}$) 5-HT concentrations when compared with vehicle treated controls (474 ± $20\ ng{\cdot}g^{-1}$; 1040 ± $103\ ng{\cdot}g^{-1}$, respectively) ($P < 0.01$ two sample *t*-test). Neither PCPA nor MDMA affected noradrenaline or dopamine concentrations in the cortex or hypothalamus (data not shown).

ANOVA of body temperature showed effects of MDMA [$F(1,28) = 35.73$, $P < 0.001$], caffeine [$F(1,28) = 3.34$, $P = 0.018$], time [$F(6168) = 42.84$, $P < 0.001$], MDMA × time [$F(6168) = 36.18$, $P < 0.001$], caffeine × time [$F(6, 168) = 3.62$, $P < 0.01$] and MDMA × caffeine × time [$F(6168) = 2.22$, $P < 0.05$]. *Post hoc* comparisons revealed that MDMA increased body temperature 0.5, 1 and 2 h following administration when compared with vehicle-treated controls. Caffeine alone did not significantly alter body temperature when compared with vehicle-treated controls. Caffeine increased MDMA-induced hyperthermia 1, 2 and 3 h following drug administration (Figure 1B).

Catecholamine depletion blocks MDMA-induced hyperthermia and its exacerbation by caffeine
αMPT and reserpine induced an 81% depletion in cortical noradrenaline concentrations (88 ± $32\ ng{\cdot}g^{-1}$) when compared with vehicle treated controls (466 ± $12\ ng{\cdot}g^{-1}$) ($P <$

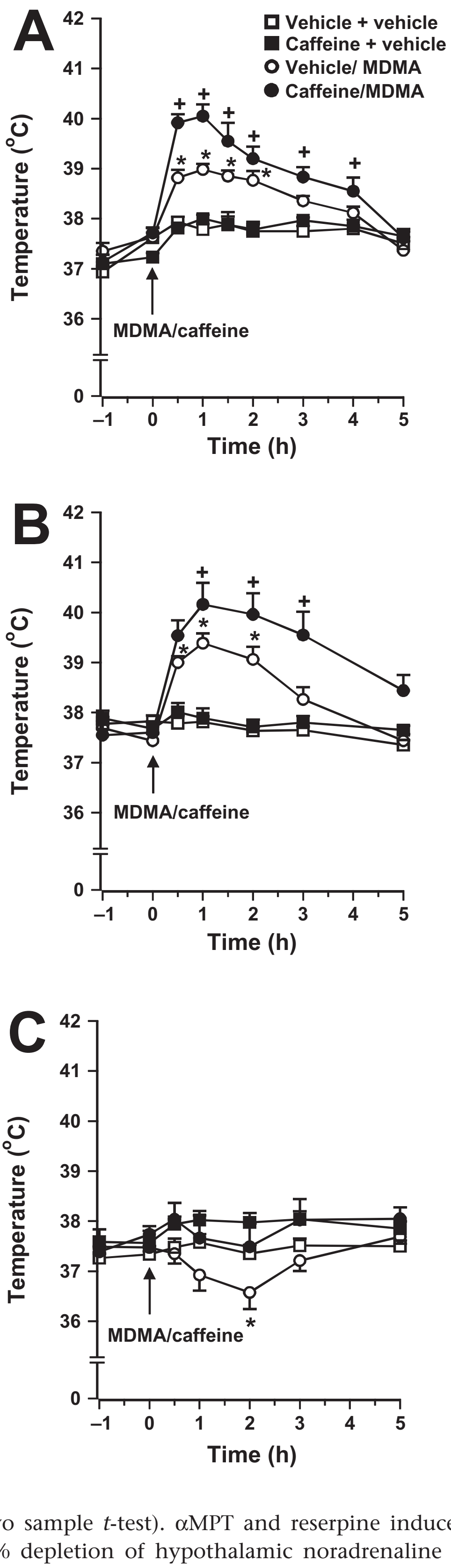

Figure 1 Catecholamine but not 5-HT depletion blocks MDMA-induced hyperthermia. There was no difference in body temperature between the groups at T0 prior to challenge. Values represent mean with standard error of the mean. *$P < 0.01$ versus vehicle control; +$P < 0.01$ versus vehicle + MDMA. (A) Caffeine potentiates MDMA-induced hyperthermia: $n = 12$ animals per group. (B) 5-HT depletion does not influence MDMA-induced hyperthermia or its exacerbation by caffeine: $n = 6–8$ animals per group. (C) Catecholamine depletion blocked MDMA-induced hyperthermia. $n = 8$ animals per group. MDMA, methylenedioxymethamphetamine.

0.01, two sample *t*-test). αMPT and reserpine induced a 72 and 68% depletion of hypothalamic noradrenaline (934 ± 180 ng·g^{-1}) and dopamine (123 ± 44 ng·g^{-1}) concentrations when compared with vehicle-treated controls (3326 ± 400 ng·g^{-1}; 387 ± 52 ng·g^{-1}, respectively) ($P < 0.01$; two sample *t*-test).

Pretreatment with αMPT and reserpine blocked MDMA-induced hyperthermia. Three-way ANOVA showed effects of caffeine [$F(1,28) = 5.52$, $P = 0.026$], time [$F(6168) = 5.42$, $P < 0.001$], MDMA × time [$F(6168) = 5.74$, $P < 0.001$] and caffeine × time [$F(6168) = 3.24$, $P = 0.005$] on body temperature. *Post hoc* comparisons revealed that MDMA decreased body temperature 2 h following administration when compared with vehicle-treated controls. Caffeine alone did not significantly alter body temperature when compared with vehicle-treated controls. MDMA did not produce hypothermia in caffeine + MDMA treated-animals when compared with MDMA treatment alone (Figure 1C).

Caffeine does not alter brain concentrations of MDMA or its major metabolite MDA

Analysis of MDMA and MDA concentrations showed an effect of time only [$F(5, 36) = 127.5$, $P < 0.0001$] and [$F(5, 36) = 157$, $P < 0.0001$], respectively. Brain concentrations of MDMA were maximal 30 min after administration, reaching 12 ng·mg^{-1} (65 nmol·g^{-1}). Peak levels fell by over 50% 2 h following administration, and only minute concentrations were quantifiable 24 h later. Brain concentrations of MDA were maximal (16 nmol·g^{-1}) 1 h after MDMA administration and had fallen by nearly a third from peak levels at 4 h. Concentrations of MDA were barely detectable 24 h following administration. Co-administration of caffeine did not significantly affect brain concentrations of MDMA or MDA following drug treatment, compared with MDMA administration alone (Figure 2A and B).

Co-administration of caffeine does not alter D-fenfluramine-induced hypothermia

Measurement of body temperature showed effects of D-fenfluramine [$F(1,28) = 90.97$, $P < 0.001$], time [$F(8224) = 3.4$, $P = 0.001$] and a D-fenfluramine-time interaction [$F(8224) = 33.07$, $P < 0.001$]. *Post hoc* comparisons revealed that D-fenfluramine reduced body temperature 1, 1.5, 2, 3 and 4 h following administration when compared with vehicle-treated controls. Caffeine alone did not significantly alter body temperature when compared with vehicle treated controls. Caffeine did not influence D-fenfluramine-induced hypothermia (Figure 3A).

Co-administration of caffeine does not alter D-amphetamine-induced hyperthermia Analysis of body temperature showed effects of D-amphetamine [$F(2,41) = 19.07$, $P < 0.001$], time [$F(8328) = 56.03$, $P < 0.001$] and D-amphetamine × time [$F(16\ 328) = 13.06$, $P < 0.001$].

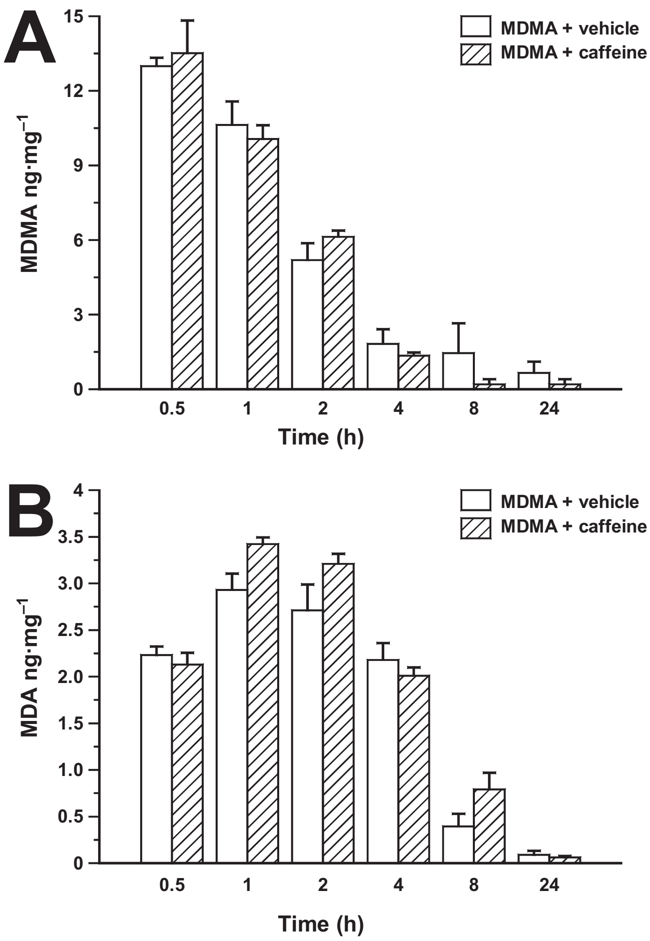

Figure 2 Influence of caffeine on the metabolism of MDMA. Brain concentrations of (A) MDMA and (B) MDA following administration of MDMA (15 mg·kg^{-1}; i.p.) alone or in combination with caffeine (10 mg·kg^{-1}; i.p.). All data expressed as mean + SEM (ng/mg of brain tissue) of four rats per group. αMPT, alpha methyl para tyrosine; MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethamphetamine.

D-amphetamine (15 mg·kg^{-1})-induced hyperthermia 0.5, 1, 1.5 and 2 h following drug administration when compared with vehicle-treated controls. Caffeine alone, or co-injected with D-amphetamine (5 or 15 mg·kg^{-1}), did not significantly alter body temperature when compared with D-amphetamine treatment alone (Figure 3B).

Co-administration of caffeine provokes D-amphetamine + D-fenfluramine-induced hyperthermia

Body temperature was affected by D-amphetamine (1 mg·kg^{-1}) + D-fenfluramine (5 mg·kg^{-1}) [F(1,28) = 14.53, P < 0.001], caffeine [F(1,28) = 33.68, P < 0.001], D-amphetamine + D-fenfluramine × caffeine [F(1,28) = 9.24, P < 0.001], time [F(8224) = 30.66, P < 0.001], caffeine × time [F(8224) = 2.08, P = 0.039], D-amphetamine + D-fenfluramine × time [F(8224) = 11.08, P < 0.001], and a D-amphetamine + D-fenfluramine × caffeine-time interaction [F(8224) = 15.91, P < 0.001]. *Post hoc* comparisons revealed that the combination of D-amphetamine (1 mg·kg^{-1}) + D-fenfluramine (5 mg·kg^{-1}) did not influence body temperature after challenge when compared with vehicle-treated controls. Caffeine alone did not increase body temperature but provoked D-amphetamine + D-fenfluramine-induced hyperthermia 30 min, 1, 1.5, 2 and 3 h after drug administration when compared with D-amphetamine + D-fenfluramine treatment alone (Figure 3C).

Co-administration of caffeine attenuates DOI-induced hypothermia

Analysis of body temperature showed effects of DOI [F(1,19) = 20.78, P < 0.001], caffeine [F(1,19) = 41.75, P < 0.001], time [F(8152) = 12.73, P < 0.001], DOI × time [F(8152) = 2.21, P = 0.029] and a DOI × caffeine-time interaction [F(8, 152) = 4.81, P < 0.001]. *Post hoc* comparisons revealed that DOI decreased body temperature 1, 1.5 and 2 h following administration when compared with vehicle-treated controls. Caffeine attenuated DOI-induced hypothermia 1, 1.5 and 2 h following drug administration (Figure 4A).

Co-administration of caffeine fails to alter apomorphine-induced hypothermia

Experiments with apomorphine and caffeine showed effects on body temperature of apomorphine [F(1,28) = 14.96, P < 0.001], caffeine [F(1,28) = 9.40, P = 0.005], time [F(8224) = 6.50, P < 0.001], apomorphine × time [F(8224) = 3.34, P = 0.001] and an apomorphine × caffeine-time interaction [F(8, 224) = 4.41, P < 0.001]. *Post hoc* comparisons revealed that apomorphine decreased body temperature 1 h following administration when compared with vehicle-treated controls. Caffeine provoked hypothermia in animals 30 min following apomorphine administration, but did not influence apomorphine-induced hypothermia 1 h following drug administration compared with apomorphine treatment alone (Figure 4B).

Caffeine provokes hyperthermia following treatment with a combination of apomorphine and DOI

ANOVA of body temperature showed effects of caffeine [F(1,19) = 21.43, P < 0.001], time [F(8152) = 21.07, P < 0.001], DOI + apomorphine × time [F(8152) = 4.68, P < 0.001], caffeine × time [F(8152) = 3.26, P = 0.002] and DOI + apomorphine × caffeine-time interaction [F(8152) = 4.22, P < 0.001]. *Post hoc* comparisons revealed that treatment with DOI + apomorphine did not alter core body temperature when compared with vehicle-treated controls. When DOI + apomorphine were co-administered with caffeine, there was an increase in body temperature 30 min, 1 and 1.5 h following administration when compared with caffeine or DOI + apomorphine-treated controls (Figure 4C).

Prior administration of SCH 23390 blocks MDMA-induced hyperthermia and its exacerbation following the co-administration of caffeine

Without SCH 23390, body temperature showed effects of MDMA [F(1,36) = 54.1. P < 0.001], caffeine [F(1,36) = 36.72, P

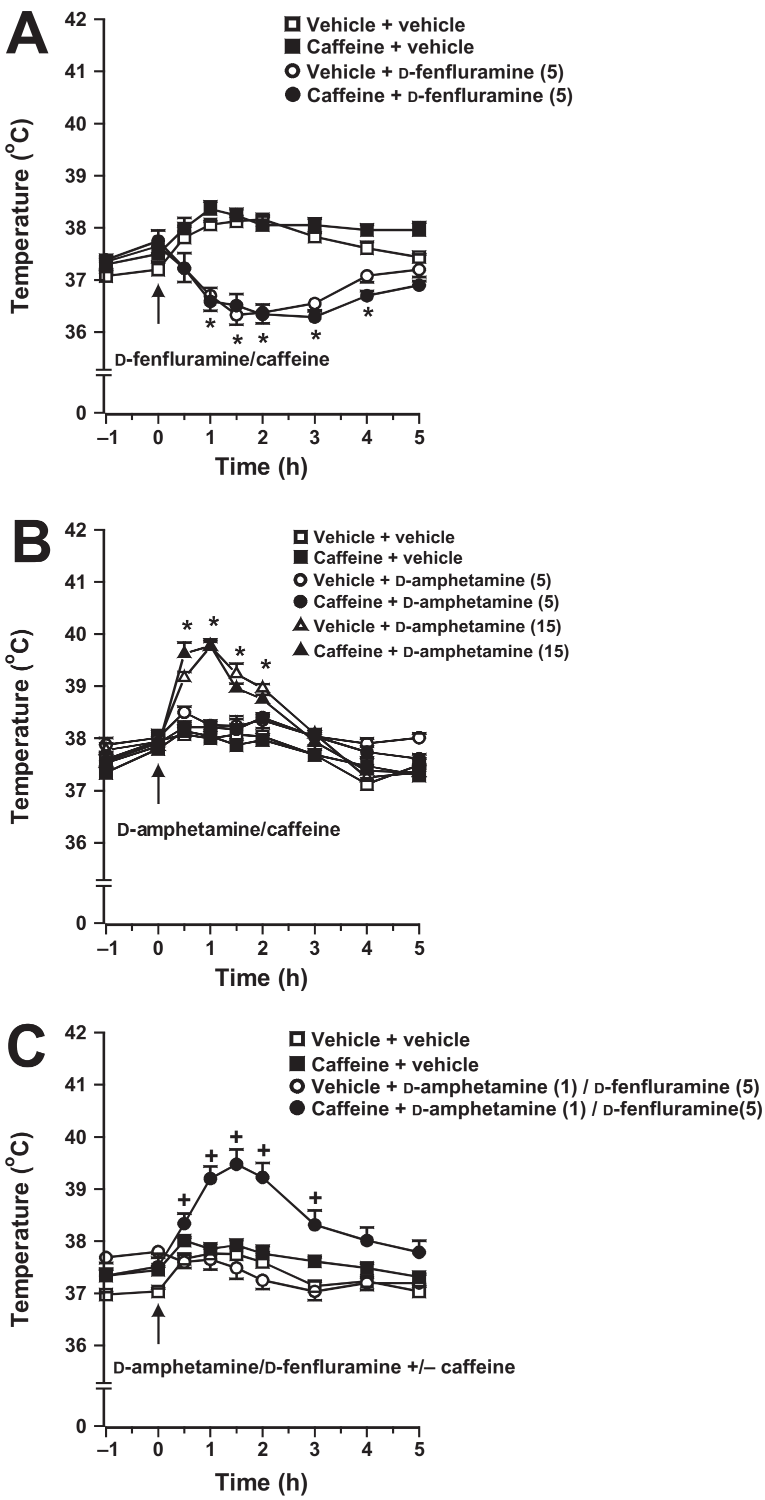

Figure 3 Caffeine fails to influence (A) D-fenfluramine-induced hypothermia (B) D-amphetamine-induced hyperthermia but (C) provokes hyperthermia following treatment with a combination of D-amphetamine (1 $mg \cdot kg^{-1}$) and D-fenfluramine (5 $mg \cdot kg^{-1}$). There was no difference in body temperature between the groups at T0 prior to challenge. Values represent mean of 7–8 rats with standard error of the mean. *$P <$ 0.01versus vehicle control; +$P < 0.01$ versus vehicle + D-fenfluramine/D-amphetamine. Numbers in parentheses represent doses in $mg \cdot kg^{-1}$.

< 0.001], time [$F(7252) = 71.90$, $P < 0.001$], MDMA × caffeine [$F(1,36) = 4.73$, $P = 0.036$], MDMA × time [$F(7252) = 4.93$, $P <$ 0.001] and caffeine × time [$F(7, 252) = 2.33$, $P < 0.001$]. *Post hoc* comparisons revealed that MDMA increased body temperature 0.5, 1 and 2 h following administration when compared with vehicle-treated controls. Caffeine alone did not significantly alter body temperature when compared with vehicle-treated controls. Caffeine increased MDMA-induced

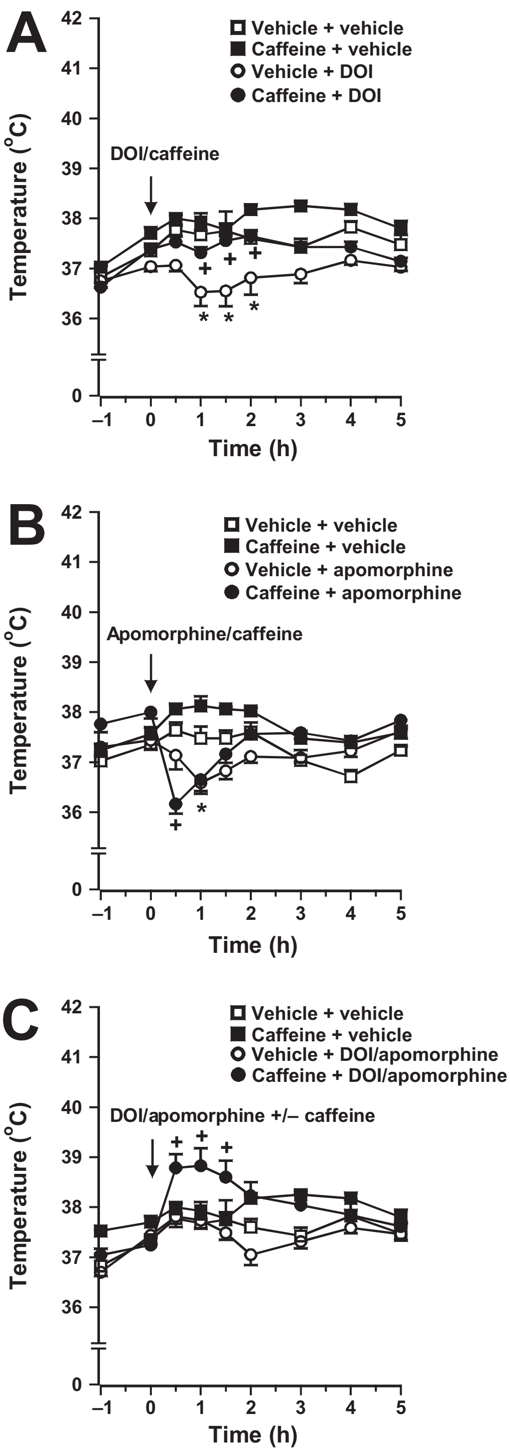

Figure 4 Caffeine fails to influence (A) DOI-induced hypothermia (B) apomorphine-induced hypothermia but (C) provokes hyperthermia following treatment with a combination of DOI and apomorphine. There was no difference in body temperature between the groups at T0 prior to challenge. Values represent mean of 4–8 rats with standard error of the mean. *$P < 0.01$ versus vehicle control, +$P < 0.01$ versus (A) vehicle + DOI (B) vehicle + apomorphine and (C)caffeine + vehicle or vehicle + DOI/Apomorphine treated groups. DOI, 2,5 dimethoxy-4-iodophenyl-aminopropane hydrochloride.

hyperthermia 0.5, 1, 2, 3 and 4 h following drug administration compared with the MDMA alone-treated group (Figure 5A).

With SCH 23390, three-way ANOVA of body temperature showed effects of MDMA [$F(1,44) = 31.64$, $P < 0.0001$], time [$F(7308) = 45.97$, $P < 0.0001$], MDMA × time [$F(7308) = 13.48$, $P < 0.0001$] and a MDMA × caffeine-time interaction [$F(7308) = 4.20$, $P < 0.001$]. Pretreatment with SCH 23390 blocked any increase in core body temperature following MDMA administration alone or in combination with caffeine. *Post hoc* comparisons revealed that MDMA induced hypothermia 1 h following drug administration. Caffeine alone did not significantly alter body temperature when administered alone or in combination with MDMA-treated groups (Figure 5B).

Prior administration of ketanserin and prazosin, but not ritanserin, blocks MDMA-induced hyperthermia and its exacerbation following the co-administration of caffeine

Ketanserin. ANOVA of body temperature showed effects of caffeine [$F(1,28) = 6.58$, $P = 0.016$], MDMA × time [$F(8224) = 4.55$, $P < 0.001$] and caffeine × time [$F(8224) = 7.66$, $P < 0.001$]. Pretreatment with ketanserin blocked MDMA-induced hyperthermia. A reduction in core body temperature was observed in the MDMA-treated group 1 h following drug administration when compared with vehicle-treated controls. Moreover, co-administration of caffeine with MDMA did not produce a change in body temperature when compared with MDMA treatment alone (Figure 5C).

Ritanserin. ANOVA of body temperature showed effects of caffeine [$F(1,31) = 5.15$, $P = 0.03$], MDMA [$F(1,31) = 73.74$, $P < 0.001$], time [$F(8248) = 129.29$, $P < 0.001$], MDMA × time [$F(8248) = 21.62$, $P < 0.001$], caffeine × time [$F(8248) = 9.18$, $P < 0.001$] and MDMA × caffeine-time interaction [$F(8248) = 3.39$, $P = 0.001$]. MDMA provoked an increase in core body temperature 0.5, 1, 1.5 and 2 h post-administration when compared with vehicle-treated controls. Co-treatment with caffeine potentiated this response 3 h following MDMA administration when compared with the MDMA alone-treated group (Figure 5D).

Prazosin. ANOVA of body temperature showed effects of caffeine [$F(1,27) = 10.99$, $P = 0.003$], time [$F(8216) = 3.69$, $P < 0.001$], caffeine × time [$F(8216) = 3.19$, $P = 0.002$] and MDMA × caffeine-time interaction [$F(8216) = 2.99$, $P = 0.003$]. Caffeine provoked an increase in core body temperature 3 h post-administration when compared with vehicle-treated controls. Caffeine provoked an increase in core body temperature in MDMA co-treated animals 2 h post-administration when compared with MDMA-treated controls. There were no other differences found between the treatment groups (Figure 5E).

Co-treatment with the adenosine receptor antagonists CGS 15943 or DPCPX fails to influence MDMA-induced hyperthermia

CGS 15943. ANOVA of body temperature showed effects of MDMA [$F(1,36) = 22.25$, $P < 0.001$], time [$F(8288) = 15.94$,

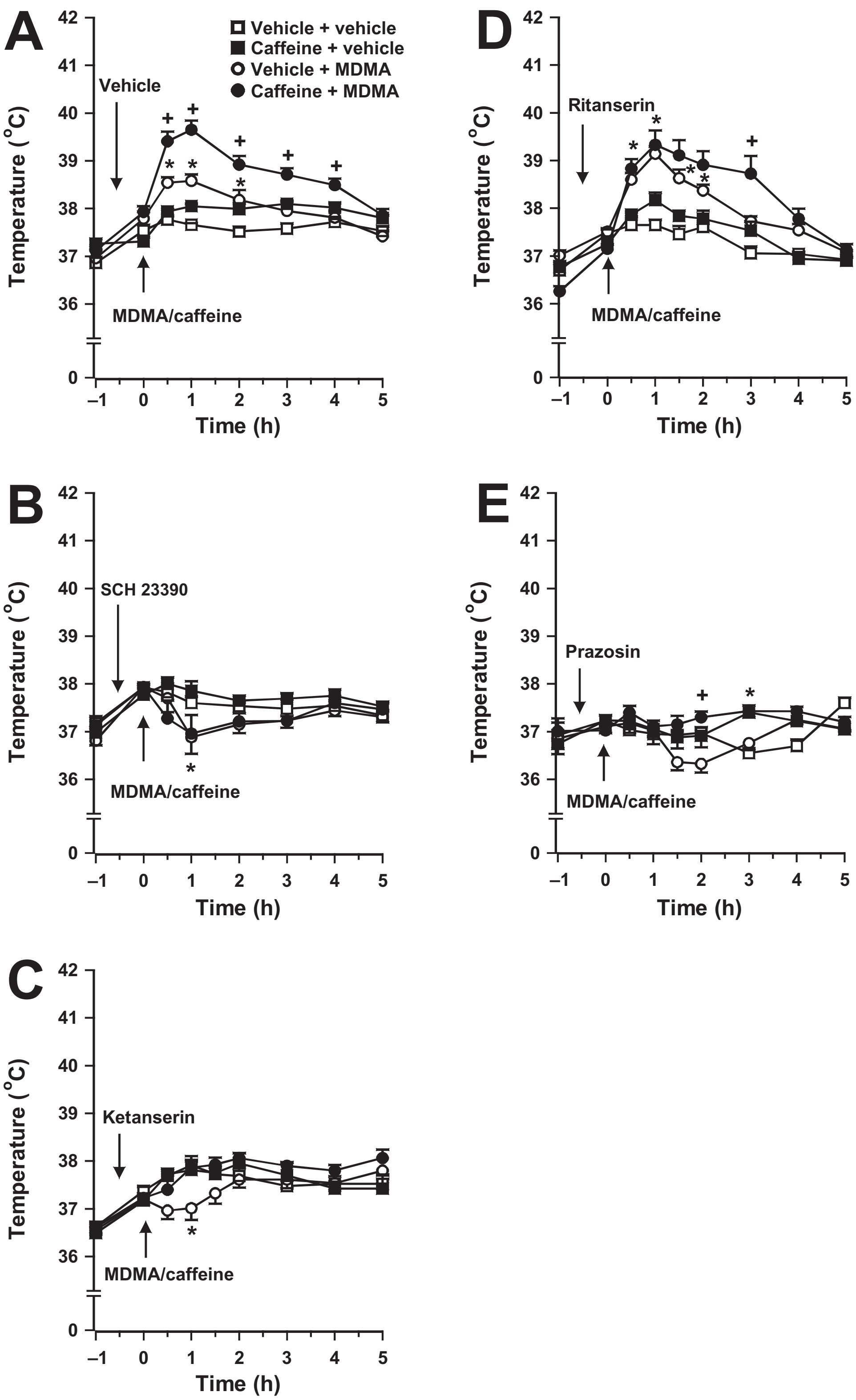

Figure 5 Influence of prior administration of dopamine D_1, 5-HT_2 and α_1 adrenoceptor antagonists on MDMA-induced hyperthermia and its exacerbation by caffeine. In each experiment, there was no difference in body temperature between the groups at T0 prior to challenge. Panel A shows the response without antagonist, and, in panel B, SCH-23390 blocks the interaction between caffeine and MDMA. Prior administration of the $5HT_{2A}$ receptor antagonists (C) ketanserin but not (D) ritanserin blocks the interaction between caffeine and MDMA. Prior administration of the α_1 adrenoceptor antagonist (E) prazosin blocks the interaction between caffeine and MDMA. Values represent mean of 7–12 rats with standard error of the mean. *$P < 0.01$ versus vehicle control. +$P < 0.01$ versus vehicle + MDMA. MDMA, methylenedioxymethamphetamine.

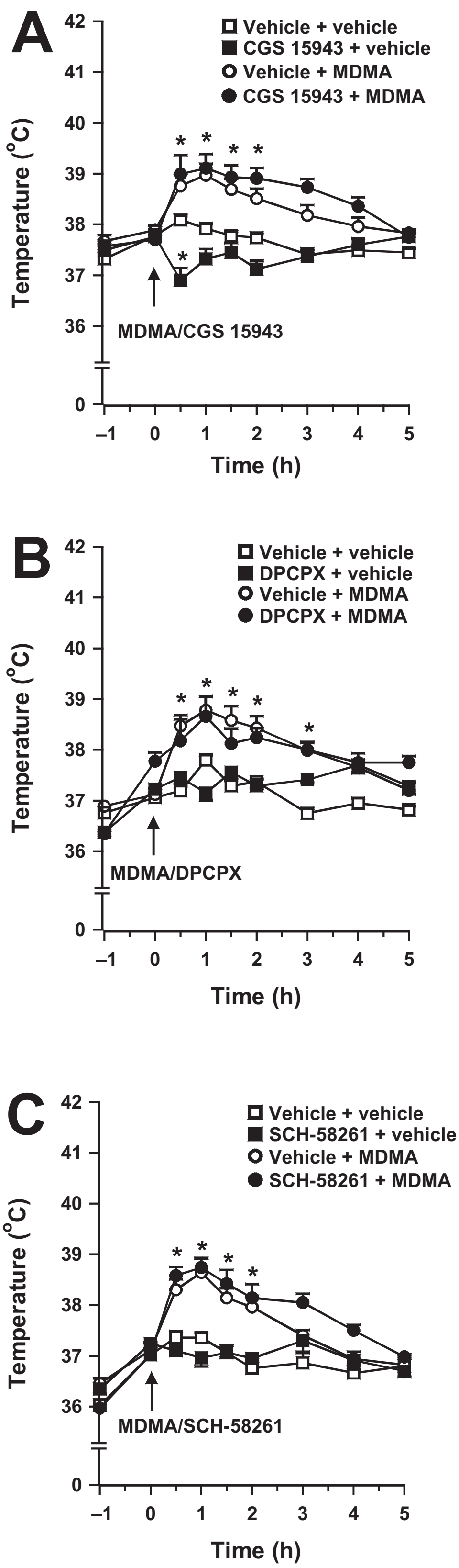

Figure 6 Co-administration of (A) 9-chloro-2-(2-furanyl)-[1,2,4] triazolo[1,5-C]quinazolin-5-amine (CGS)-15943 (B) DPCPX and (C) schering (SCH) 58261 fail to alter MDMA-induced hyperthermia. There was no difference in body temperature between the groups at T0 prior to challenge. Values represent mean of 5–10 rats with standard error of the mean. *P < 0.01 versus vehicle control group. DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; MDMA, methylenedioxymethamphetamine.

P < 0.001] and a CGS × MDMA-time interaction [F(8288) = 5.20, P < 0.001]. MDMA induced hyperthermia 0.5, 1, 1.5 and 2 h following drug administration when compared with vehicle-treated controls. CGS alone reduced body temperature 30 min post-challenge when compared with vehicle-treated controls. Co-administration of CGS with MDMA did not significantly alter body temperature when compared with MDMA treatment alone (Figure 6A).

DPCPX. Three-way ANOVA of body temperature showed effects of MDMA [F(1,32) = 56.69, P < 0.001], time [F(8256) = 40.18, P < 0.001] and a DPCPX × MDMA-time interaction [F(8256) = 2.76, P = 0.006]. MDMA induced hyperthermia 0.5, 1, 1.5, 2 and 3 h following drug administration when compared with vehicle-treated controls. DPCPX did not influence body temperature when compared with vehicle-treated controls. Co-administration of DPCPX with MDMA did not significantly alter body temperature when compared with MDMA treatment alone (Figure 6B).

SCH 58261. Three-way ANOVA of body temperature showed effects of MDMA [F(1,19) = 52.86, P < 0.001], time [F(8152) = 67.88, P < 0.001], MDMA × time [F(8152) = 19.35, P < 0.001], SCH × time [F(8152) = 3.33, P = 0.002] and a SCH × MDMA-time interaction [F(8152) = 2.14, P = 0.035]. MDMA induced hyperthermia 0.5, 1, 1.5 and 2 h following drug administration when compared with vehicle-treated controls. Co-administration of SCH with MDMA did not significantly alter body temperature when compared with MDMA treatment alone (Figure 6C).

Co-treatment with the PDE inhibitors pentoxyfylline, rolipram or zaprinast fails to influence MDMA-induced hyperthermia

Pentoxyfylline. ANOVA of body temperature showed effects of MDMA [F(1,32) = 17.53, P < 0.001] and time [F(8256) = 32.43, P < 0.001]. MDMA induced hyperthermia 0.5 and 1 h following drug administration when compared with vehicle-treated controls. Pentoxyfylline alone did not influence body temperature when compared with vehicle-treated controls. Co-administration of pentoxyfylline with MDMA did not significantly alter body temperature when compared with MDMA treatment alone (Figure 7A).

Rolipram. Three-way ANOVA of body temperature showed effects of MDMA [F(1,32) = 52.44, P < 0.001], time [F(8256) = 18.21, P < 0.001] and a rolipram × MDMA-time interaction [F(8256) = 15.55, P < 0.001]. MDMA induced hyperthermia 0.5, 1, 1.5 and 2 h following drug administration when compared with vehicle-treated controls. Rolipram alone reduced

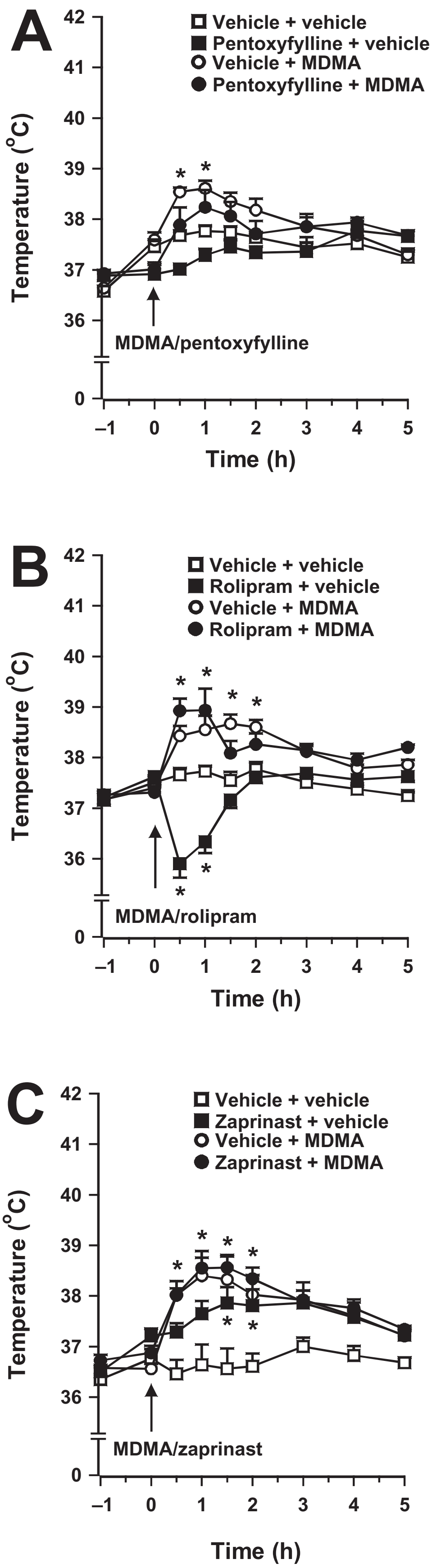

Figure 7 Co-administration of (A) pentoxyfylline (B) rolipram or (C) zaprinast fail to influence MDMA-induced hyperthermia. There was no difference in body temperature between the groups at T0 prior to challenge. Values represent mean of 8–10 rats with standard error of the mean. $^*P < 0.01$ versus vehicle control. MDMA, methylenedioxymethamphetamine.

body temperature 30 min and 1 h following challenge when compared with vehicle-treated controls. Co-administration of rolipram with MDMA did not significantly alter body temperature when compared with MDMA treatment alone (Figure 7B).

Zaprinast. Three-way ANOVA of body temperature showed effects of MDMA [$F(1,32) = 21.16$, $P < 0.001$], zaprinast [$F(1,32) = 10.94$, $P = 0.002$] and time [$F(8256) = 34.16$, $P < 0.001$]. MDMA induced hyperthermia 30 min, 1, 1.5 and 2 h following drug administration when compared with vehicle-treated controls. Zaprinast alone increased body temperature 1.5 and 2 h following drug administration when compared with vehicle-treated controls. Co-administration of zaprinast with MDMA did not significantly alter body temperature when compared with MDMA treatment alone (Figure 7C).

Co-treatment with rolipram and CGS 15943 or SCH 58261 but not DPCPX exacerbate MDMA-induced hyperthermia

CGS 15943 with rolipram. ANOVA of body temperature showed effects of CGS/rolipram [$F(1,28) = 173.4$, $P < 0.001$], MDMA [$F(1,28) = 13.36$, $P = 0.001$], a CGS/rolipram-MDMA interaction [$F(1,28) = 14.23$, $P < 0.001$], time [$F(8224) = 60.85$, $P < 0.001$] and CGS/rolipram × time [$F(8224) = 40.89$, $P < 0.001$], MDMA × time [$F(8224) = 2.42$, $P = 0.016$] and CGS/rolipram × MDMA-time interactions [$F(8,24) = 10.43$, $P < 0.001$]. MDMA induced hyperthermia 0.5, 1, 1.5, 2 and 3 h following drug administration when compared with vehicle-treated controls. CGS/rolipram alone did not influence body temperature when compared with vehicle-treated controls. Co-administration of CGS/rolipram with MDMA potentiated MDMA-induced hyperthermia 0.5, 1, 1.5, 2 and 3 h post administration when compared with MDMA treatment alone (Figure 8A).

DPCPX with rolipram. ANOVA of body temperature showed effects of MDMA [$F(1,26) = 75.59$, $P < 0.001$], time [$F(8208) = 51.03$, $P < 0.001$], MDMA × time [$F(8208) = 65.31$, $P < 0.001$], and DPCPX/rolipram × time [$F(8208) = 9.20$, $P < 0.001$] and DPCPX/rolipram × MDMA-time interactions [$F(8208) = 10.07$, $P < 0.001$]. MDMA induced hyperthermia 0.5, 1, 1.5, 2 and 3 h following drug administration when compared with vehicle-treated controls. DPCPX/rolipram alone reduced body temperature 0.5 and 1 h following challenge when compared with vehicle-treated controls. Co-administration of DPCPX/rolipram with MDMA did not significantly alter body temperature when compared with MDMA treatment alone (Figure 8B).

SCH 58261 with rolipram. ANOVA of body temperature showed effects of MDMA [$F(1,18) = 68.09$, $P < 0.001$], SCH/

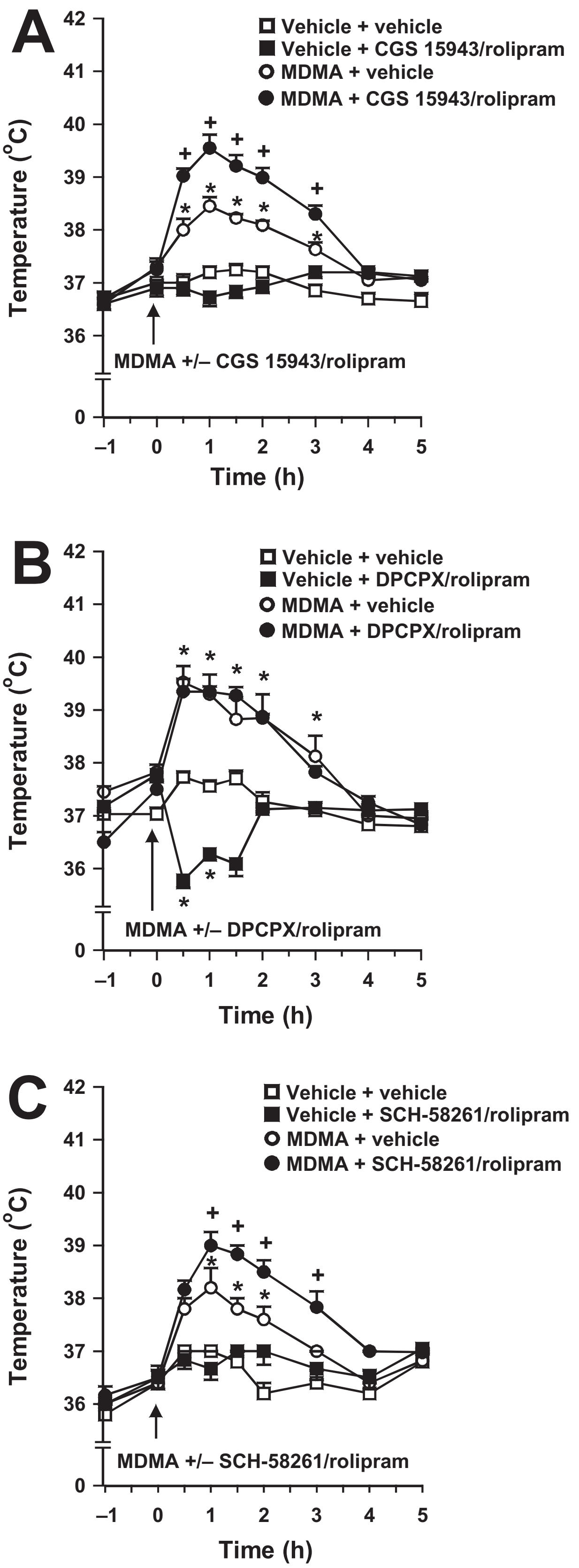

Figure 8 Rolipram in combination with (A) 9-chloro-2-(2-furanyl)-[1,2,4]triazolo[1,5-C]quinazolin-5-amine (CGS) 15943 (C) schering (SCH) 58261 but not (B) DPCPX exacerbates MDMA-induced hyperthermia. There was no difference in body temperature between the groups at T0 prior to challenge. Values represent mean of 5–8 rats with standard error of the mean. *$P < 0.01$ versus vehicle control group. +$P < 0.01$ versus MDMA + vehicle-treated group. DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; MDMA, methylenedioxymethamphetamine.

rolipram [$F(1,18) = 12.12$, $P = 0.003$], an SCH/rolipram-MDMA interaction [$F(1,18) = 4.46$, $P < 0.05$], time [$F(8144) = 68.53$, $P < 0.001$], and SCH/rolipram × time [$F(8144) = 22.04$, $P < 0.001$], MDMA × time [$F(8144) = 2.49$, $P = 0.014$] and SCH/rolipram × MDMA-time interactions [$F(8144) = 2.49$, $P = 0.014$]. MDMA induced hyperthermia 1, 1.5 and 2 h following drug administration when compared wirh vehicle-treated controls. SCH/rolipram alone did not influence body temperature when compared with vehicle-treated controls. Co-administration of SCH/rolipram with MDMA potentiated MDMA-induced hyperthermia 1, 1.5, 2 and 3 h post-administration when compared with MDMA treatment alone (Figure 8C).

Discussion

Caffeine exacerbates MDMA-induced hyperthermia but does not influence brain concentrations of MDMA or its metabolite MDA

In line with our previous observations, caffeine exacerbates MDMA-induced hyperthermia in rats (McNamara *et al.*, 2006). Since caffeine is a well-known inhibitor of CYP 1A2 (Carrillo and Benitez, 2000; Kot and Daniel, 2008), and N-demethylation of MDMA to methylenedioxyamphetamine (MDA) may be catalyzed in rats and humans by CYP 1A2 (Maurer *et al.*, 2000), it was conceivable that altered metabolism of MDMA by caffeine might lead to a higher-than-usual plasma concentration and account for the observed effect on core body temperature. However, co-administration of caffeine with MDMA did not influence the concentration of MDMA or its metabolite MDA in the brain. MDMA also failed to alter the concentration of caffeine or its metabolites paraxanthine, theophylline and theobromine in the brain (data not shown). As caffeine did not alter MDMA and MDA concentrations in the brain, we propose that the interaction between the drugs is pharmacodynamic in nature.

A role for catecholamines and 5-HT in the interaction

The present report provides evidence for a dual role of 5-HT and catecholamines in MDMA-induced hyperthermia and its exacerbation by caffeine. Initially, depletion of endogenous catecholamines attenuated MDMA-induced hyperthermia. Furthermore, a specific role for dopamine is suggested as pre-treatment with the dopamine D_1 receptor antagonist, SCH 23390, attenuated MDMA-induced hyperthermia and inhibited the ability of caffeine to exacerbate MDMA-induced hyperthermia. By contrast, depletion of central 5-HT failed to block MDMA-induced hyperthermia and exacerbation by caffeine. To further understand 5-HT and catecholaminergic mechanisms mediating the hyperthermic response to MDMA and its exacerbation by caffeine, we examined the effect of caffeine on the core body temperature response to two other amphetamines. D-fenfluramine induces hypothermia in rats due to its selective interactions with the 5-HT system (Cryan *et al.*, 1999), whereas D-amphetamine increases core body temperature in rats (Jaehne *et al.*, 2005) via catecholamine-dependent mechanisms (Glaser *et al.*, 2005). We first determined if co-administration of caffeine with D-fenfluramine would provoke hyperthermia. Caffeine failed to alter the hypothermic response to D-fenfluramine, suggesting that 5-HT release does not play a role in mediating the ability of caffeine to promote hyperthermia. However, upon further exploration of the mechanisms, we observed that caffeine failed to increase D-amphetamine-induced hyperthermia and provoked an MDMA-like response only when D-amphetamine was co-administered with D-fenfluramine. Such a response indicates that, despite the lack of interaction following 5-HT depletion, 5-HT release is nevertheless an important contributing factor to the interaction between caffeine and MDMA.

As the 5-HT system plays a major role in mammalian thermoregulation and is one of the primary targets of the substituted amphetamines, it has often been assumed that this neurotransmitter is responsible for the hyperthermia seen following MDMA administration (Shankaran and Gudelsky, 1999). This theory is supported by studies showing that 5-HT$_2$ receptor antagonists can prevent MDMA-induced hyperthermia (Nash *et al.*, 1988; Schmidt *et al.*, 1990). More recently however, Mechan *et al.* (2002) suggests that 5-HT may not be such a key player in MDMA-induced hyperthermia, as pretreatment with the 5-HT$_2$ receptor antagonists methysergide, MDL 100,907, SB 242084 and ritanserin or the 5-HT re-uptake inhibitors zimeldine and fluoxetine failed to influence MDMA-induced hyperthermia in rats. The present study also suggests that 5-HT does not play a primary role in either MDMA-induced hyperthermia or its exacerbation by caffeine, as central depletion of 5-HT did not influence the response to drug challenge. Conversely, catecholamine depletion blocked the hyperthermia and provoked a switch to hypothermia in response to MDMA. A similar finding has been previously reported by Dafters and Biello (2003), where acute co-administration of αMPT or the dopamine receptor antagonist, haloperidol, reversed MDMA-induced hyperthermia to produce a hypothermic response. Interestingly, despite the profound effect of catecholamine depletion on MDMA-induced hyperthermia here, it did not fully prevent the interaction between caffeine and MDMA. As reserpine and αMPT treatment only resulted in ~70% depletion in noradrenaline and dopamine, it is possible that the remaining catecholamine content was sufficient to mediate an interaction between caffeine and MDMA.

Dopamine is involved in thermoregulation, especially in the pre-optic area and anterior hypothalamus, which are the primary loci for maintenance of body temperature (see Hasegawa *et al.*, 2005). In this regard, both dopamine D$_1$ and D$_2$ receptor subtypes are implicated in MDMA-induced changes in body temperature. MDMA-induced hypothermia in rats housed at 15°C can be blocked by pretreatment with the dopamine D$_2$ receptor antagonist remoxipride, but not the dopamine D$_1$ receptor antagonist SCH 23390 (Green *et al.*, 2005). This is the converse of the hyperthermic response, which is blocked by SCH 23390 but unaltered by remoxipride (Mechan *et al.*, 2002). It has been proposed that dopamine D$_2$ receptor stimulation predominates in animals housed individually or at low ambient temperatures, which is why hypothermia is observed in such animals in response to MDMA. We have previously described how co-administration of caffeine switches the hypothermic response to MDMA in individually housed animals to a profound hyperthermia (McNamara *et al.*, 2006). Under such conditions caffeine, may override dopamine D$_2$ receptor-mediated hypothermia, and promote a switch to D$_1$ receptor-mediated hyperthermia.

Such a mechanism is consistent with a number of the responses obtained following drug challenge in the current study. MDMA provoked a hypothermic response following catecholamine depletion or dopamine D$_1$ receptor blockade. Dopamine itself has a greater affinity for dopamine D$_2$-like receptors, in particular D$_3$ and D$_4$ (Missale *et al.*, 1998), and it is possible that in the depletion study, although there was insufficient dopamine release to provoke an overt dopamine D$_1$ receptor-mediated hyperthermic response to MDMA, a D$_2$-like hypothermia occurred instead. Co-administration with caffeine may overcome the dopamine D$_2$ receptor-mediated response by amplifying the dopamine signal. However, a switch to hyperthermia is not obtained in animals pretreated with SCH 23390, as dopamine D$_1$ receptors are blocked under these conditions. Similar responses were obtained where the combination of D-fenfluramine and D-amphetamine provoked a hypothermic response following dopamine D$_1$ receptor blockade (data not shown), and co-administration of caffeine failed to provoke a switch to hyperthermia. As MDMA provokes the release of dopamine in the brain (see Green *et al.*, 2003 for review), and caffeine has also been reported to influence central dopamine release (see Cauli and Morelli, 2005; Ferré, 2008), dopamine release may represent a mechanism whereby caffeine effects a change from a D$_2$ to a D$_1$ receptor-mediated response. Co-administration of caffeine with MDMA may provoke dopamine release sufficient to induce a switch from a D$_2$ receptor mediated hypothermic response to a D$_1$ receptor-mediated hyperthermic response due to the enhanced availability of dopamine in the synapse. Further work, however, is required to clarify such a mechanism.

Despite clear effects obtained following catecholamine depletion and dopamine D$_1$ receptor blockade, and the lack of effect following central 5-HT depletion, a role for 5-HT cannot be ruled out. This is evident where the co-administration of caffeine with a combination of D-fenfluramine and D-amphetamine provokes a hyperthermic response, akin to that described following the co-administration of caffeine with MDMA (McNamara *et al.*, 2006). A role for 5-HT is further supported by evidence that pretreatment with the preferential 5-HT$_2$ receptor antagonist ketanserin attenuates the hyperthermic response to MDMA and its exacerbation by caffeine, and that co-administration of the 5-HT- and dopamine-selective agonists, DOI and apomorphine, respectively, with caffeine, provokes hyperthermia, but not when either agonist is administered with caffeine alone. MDMA has direct agonist actions at 5-HT receptors, which may account for its ability to provoke toxicity in the absence of endogenous 5-HT. Such actions include the ability of pre-synaptic 5-HT receptors to influence dopamine release and thereby augment dopamine-mediated responses to MDMA (Doly *et al.*, 2008; Gudelsky and Yamamoto, 2008). 5-HT_2 receptors play an important role in the regulation of central dopaminergic function (see Di Matteo *et al.*, 2008). It is therefore not unreasonable to suggest that ketanserin may act to reduce MDMA-induced dopamine release, resulting in the attenuation of MDMA-induced hyperthermia. Conversely, the effect of DOI may be accounted for via an enhancement of central dopamine release.

As ketanserin is also known to interact with α_1 adrenoreceptors, and α_1 blockade rather than 5-HT_2 blockade by ketanserin has been implicated in the physiological actions of ketanserin (Orallo *et al.*, 2000; Centurión *et al.*, 2006), including MDMA-induced hyperthermia (Mechan *et al.*, 2002), we examined the effects of pretreatment with the selective 5-HT_2 receptor antagonist ritanserin and the α_1 adrenoreceptor antagonist prazosin. Prior administration of ketanserin and prazosin, but not ritanserin, blocks MDMA-induced hyperthermia and its exacerbation by caffeine, suggesting that α_1-adrenoceptor blockade plays a significant role in mediating the actions of ketanserin. In support, there is substantial evidence that noradrenaline mediates MDMA-induced hyperthermia via both peripheral and central mechanisms (Bianco *et al.*, 1988; Sprague *et al.*, 2004).

A role for adenosine receptors and PDE inhibition

Under normal physiological conditions, the mechanism of action of caffeine is primarily via antagonism of adenosine receptors (Fredholm *et al.*, 1999; Fisone *et al.*, 2004; Ferre *et al.*, 2008). Modulation of dopamine transmission through adenosine receptors has been implicated in the psychostimulant effects of caffeine (Fuxe *et al.*, 1998; Cauli and Morelli, 2005), and represents a putative mechanism whereby caffeine exacerbates MDMA-induced toxicity. Antagonistic A_1-D_1 and A_{2A}-D_2 hetereomeric receptor complexes reduce dopamine receptor recognition, coupling and signalling in the basal ganglia. Moreover, caffeine is proposed to influence dopamine release via an adenosine A_1 receptor-mediated mechanism (Solinas *et al.*, 2002; Quarta *et al.*, 2004; Cauli and Morelli, 2005). In studies conducted to date, co-treatment with adenosine antagonists failed to provoke a caffeine-like interaction with MDMA, indicating that blockade of adenosine receptors alone does not mediate the interaction between caffeine and MDMA. While it has been reported that the inhibitory effect of caffeine on PDE is of little relevance at the concentrations of caffeine administered *in vivo* (Fredholm *et al.*, 1999), the weak PDE inhibiting properties of caffeine might well be relevant against a background of increased intracellular cAMP/cGMP availability following MDMA-induced biogenic amine release in the brain. Dulloo and co-workers have extensively investigated the effects of caffeine on thermogenesis induced by ephedrine. Like MDMA, ephedrine stimulates catecholamine release, its primary effect being on noradrenaline, and caffeine exacerbates ephedrine-induced hyperthermia. Following a study of the mechanisms mediating the ability of caffeine to influence the thermogenic effects of ephedrine, PDE inhibition and not adenosine receptor antagonism resulted in a potentiation of the effects of ephedrine (Dulloo *et al.*, 1991, 1992, 1994). In the current investigation however, similar to the adenosine receptor antagonists tested, co-treatment with PDE inhibitors failed to provoke a caffeine-like interaction with MDMA, indicating that inhibition of PDE alone is unlikely to mediate the interaction between caffeine and MDMA. The lack of interaction between MDMA and the PDE-5 inhibitor zaprinast is in line with a previous study reporting that treatment with the PDE-5 inhibitor sildenafil failed to influence MDMA-induced hyperthermia in rats (Puerta *et al.*, 2009). In a final step, to more fully simulate the pharmacology of caffeine, we combined treatment of the adenosine receptor antagonists with the PDE inhibitor rolipram and report that co-treatment with a low dose of the PDE-4 inhibitor rolipram and the non-selective adenosine receptor antagonist CGS 15943, or the selective adenosine A_{2A} receptor antagonist SCH 58261, exacerbate MDMA-induced hyperthermia. Thus, inhibition of PDE coupled to adenosine A_{2A} receptor blockade provokes a caffeine-like interaction with MDMA, suggesting that these targets mediate the ability of caffeine to exacerbate MDMA-induced hyperthermia.

Although the main mechanisms of action of caffeine are adenosine receptor antagonism and PDE inhibition, caffeine has also been found to increase calcium release from the sarcoplasmic reticulum through an interaction with the ryanodine receptor. Intracellular calcium release can itself induce hyperthermia and occurs in drug-induced malignant hyperthermia (Penner and Neher, 1989; Fiege *et al.*, 2002). Such a mechanism may be relevant in light of human studies, which have reported that MDMA intoxication and hyperthermia is associated with an elevation in myoplasmic calcium concentrations (Denborough and Hopkinson, 1997). In the present study, circulating concentrations of caffeine at their peak following drug administration were between 30 and 40 μM. Circulating caffeine concentrations following caffeine ingestion in humans rarely exceeds 100 μM. While caffeine can mobilize intracellular calcium, such a mechanism is unlikely to be applicable either to human consumption or in the current study as a minimal concentration of 250 μM is necessary to generate detectable effects on calcium shifts (see Nehlig *et al.*, 1992). Moreover, caffeine at the dose given did not induce significant hyperthermia, although the possibility remains that co-administration of caffeine with MDMA could influence the ability of caffeine to provoke calcium release. However, as structurally related xanthines, which influence the ryanodine receptor (see Xu *et al.*, 1998), including DPCPX and pentoxyphylline, failed to influence MDMA-induced hyperthermia, it is likely that the principal mechanism by which caffeine influences MDMA-induced hyperthermia is via the proposed mechanism involving the inhibition of adenosine A_{2A} receptors coupled to the inhibition of PDE.

In conclusion, the results of this study show that caffeine enhances the hyperthermic response to drugs that target both 5-hydroxytryptaminergic and catecholaminergic transmission but not where either system is targeted alone. Such a mechanism may account for the ability of caffeine to more readily exacerbate the acute toxicity of MDMA when compared with other amphetamines. The ability of caffeine to exacerbate MDMA-related hyperthermia may be related to inhibitory actions on adenosine A_{2A} receptors and PDE-4. Determination of the mechanisms mediating the toxicity associated with co-ingestion of caffeine with MDMA is an important step toward the treatment of severe hyperthermic reactions to ecstasy that can occur in some users. In accordance with our results and due to the fact that agents such as prazosin and ketanserin are available for human therapy, such agents or similar may be useful candidates for testing in the treatment of hyperthermia associated with MDMA ingestion alone or in combination with caffeine.

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Statement of conflicts of interest

None.

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