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A role for adenosine A₁ receptor blockade in the ability of caffeine to
promote MDMA “Ecstasy”-induced striatal dopamine release

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Summary

Co-administration of caffeine profoundly enhances the acute toxicity of 3,4-methylenedioxymethamphetamine (MDMA) in rats. The aim of this study was to determine the ability of caffeine to impact upon MDMA-induced dopamine release in superfused brain tissue slices as a contributing factor to this drug interaction. MDMA (100 and 300 μ M) induced a dose-dependent increase in dopamine release in striatal and hypothalamic tissue slices preloaded with [3 H] dopamine (1 μ M). Caffeine (100 μ M) also induced dopamine release in the striatum and hypothalamus, albeit to a much lesser extent than MDMA. When striatal tissue slices were superfused with MDMA (30 μ M) in combination with caffeine (30 μ M), caffeine enhanced MDMA-induced dopamine release, provoking a greater response than that obtained following either caffeine or MDMA applications alone. The synergistic effects in the striatum were not observed in hypothalamic slices. As adenosine A₁ receptors, one of the main pharmacological targets of caffeine, are known to play an important role in the regulation of dopamine release, their role in the modulation of MDMA-induced dopamine release was investigated. 1 μ M 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a specific A₁ antagonist, like caffeine, enhanced MDMA-induced dopamine release from striatal slices while 1 μ M 2-chloro-N(6)-cyclopentyladenosine (CCPA), a selective adenosine A₁ receptor agonist, attenuated this. Treatment with either SCH 58261, a selective A_{2A} receptor antagonist, or rolipram, a selective PDE-4 inhibitor, failed to reproduce a caffeine-like effect on MDMA-induced dopamine release. These results suggest that caffeine regulates MDMA-induced dopamine release in striatal tissue slices, via inhibition of adenosine A₁ receptors.

Keywords: MDMA, Caffeine, Superfusion, Adenosine A₁ receptors, Dopamine, Rat.

1. Introduction

Caffeine is the most widely consumed psychoactive substance. It is frequently consumed by recreational drug users with other psychostimulant drugs such as amphetamine and cocaine and has been shown to influence their toxicity (Derlet et al., 1992; Gasior et al., 2000; Poleszak et al., 2000). We and others have reported that caffeine exacerbates the acute toxicity of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) in rats, increasing lethality, and provoking hyperthermia, tachycardia and long-term 5-HT loss associated with MDMA administration (Camarasa et al., 2006; McNamara et al., 2006; 2007).

The mechanisms underlying the ability of caffeine to exacerbate MDMA-induced toxicity are not currently understood. In this regard we have recently reported that depletion of central catecholamines but not serotonin attenuates MDMA-induced hyperthermia and its exacerbation by caffeine. In addition, prior treatment with the dopamine D₁ receptor antagonist, SCH-23390, attenuates the hyperthermic response associated with this drug combination (Vanattou-Saïfoudine et al., 2010)

As MDMA provokes the release of dopamine in the brain (Green et al., 1995; El-Mallack et al., 2007) and caffeine also influences central dopamine release (Antoniou et al., 1998; Cauli et al., 2005; Quarta et al., 2004), dopamine release may represent a mechanism whereby caffeine augments the acute toxicity of MDMA. Thus, the aim of this study was to determine the effect of caffeine on MDMA-induced dopamine release in superfused tissue slices pre-loaded with [³H] dopamine. This study was performed on tissue slices obtained from two brain regions implicated in the behavioural and physiological effects of MDMA: the striatum, a key region of the motor circuitry and harbor of nerve terminal dopamine release and the

hypothalamus where dopaminergic neurons important in thermoregulation are located (You et al., 2001; De Saint Hilare et al., 2001; Fetissov et al., 2000).

There are biochemical mechanisms by which caffeine may interact with dopamine receptors or alter dopamine release. Specifically, caffeine is a non selective adenosine A₁ and A_{2A} receptor antagonist (Nehlig, 1999; Fredholm et al., 1999a and b) and negative interaction between adenosine A₁ receptors and dopamine D₁ has been described in basal ganglia (striatum, globus pallidus and substantia nigra reticulata) and limbic regions (ventral pallidum and nucleus accumbens) (Wood et al., 1989; Ballarin et al., 1995; Mayfield et al., 1999; Florán et al., 2002; Franco et al., 2007). Adenosine A₁ receptors, localised on the terminals of glutamatergic and dopaminergic neurons, can inhibit dopamine release in the brain particularly in the striatum (Borycz et al., 2007; Jin et al., 1993; O'Neill et al., 2007; Quarta et al., 2004; Wood et al., 1989).

As adenosine A₁ receptors are directly involved in regulating pre-synaptic dopamine release, we determined if blockade of adenosine A₁ receptors might simulate the actions of caffeine on MDMA-induced dopamine release in tissue slices. Our results show regionally dependent effects of caffeine on MDMA-induced dopamine release which are simulated by application of the selective adenosine A₁ receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).

2. Materials and Methods

2.1 Animals

Male Sprague Dawley rats (200-250g upon arrival) were obtained from Harlan laboratories UK. Animals were group housed under standard laboratory conditions, with a 12 hour light: 12 hour dark cycle (lights on from 08.00 to 20.00) and a temperature-controlled room maintained at 20-24°C for 2 weeks. Animals were allowed free access to commercial pellet food and tap water. All procedures were approved by the Animal Ethics Committee Trinity College Dublin and were in accordance with the European Council Directive 1986 (86/806/EEC).

2.2 Superfusion technique

Rats were rendered unconscious by stunning and following decapitation, the brain was rapidly removed, placed on an ice cold plate and the hypothalamus and striatum were dissected. These brain regions were sliced (750µm) with a McIlwain tissue chopper. Slices were suspended in 1ml of oxygenated Krebs buffer (95% O₂/5% CO₂) comprising 122mM NaCl, 3.1mM KCl, 0.4mM KH₂PO₄, 1.2mM MgSO₄(H₂O), 25mM NaHCO₃, 10mM Glucose, 1.3mM CaCl₂, 10µM pargyline adjusted to pH 7.3 and slices were subjected to gentle agitation (Stuart Gyrorocker, SSL3, 70 revolutions per minute).. Following a 30 min pre-incubation period at 37° C, the slices were incubated with 1µM [³H] dopamine (DA) (49 Ci [1.813 x 10¹²Bq] /mmol; GE Healthcare) for a further 30 min.. The preloaded slices were then transferred to six superfusion chambers of a Brandel superfusion system (3 slices/chamber) bound by polyethylene filters discs and superfused continuously (0.25ml/min) for 1h with oxygenated Krebs superfusion buffer at 37°C for equilibration

followed by the collection of four consecutive 4 min fractions of eluate to determine basal [^3H]-DA outflow (Baseline). Over the following 32 min, slices were exposed to Krebs buffer containing different concentrations alone or in combination. After this drug exposure period, the slices were superfused with drug free superfusate for an additional 12 min until [^3H] dopamine outflow returned to baseline. The radioactivity in the eluate fractions as well as the residual radioactivity in the tissue slices at the end of the experiment was determined by liquid scintillation spectroscopy using a Packard 2100 Tri-Carb liquid scintillation analyzer. The amount of labelled dopamine taken up by tissue slices was 128 ± 24.1 pmol/slice for striatal and 3.05 ± 0.72 pmol/slice for hypothalamic tissue slices.

2.3 Calculation of fractional release

To correct for variability in the amount of tissue in each superfusion chamber, radioactivity in each eluate fraction was expressed as a percentage of the total amount of radioactivity present in the slices and the filters determined at the end of the experiment. For determination of dopamine release, a baseline was calculated by averaging samples collected prior to drug exposure and this mean was subtracted from all values to determine change from the baseline average. Area under the curve (AUC) was determined by summation of the changes from baseline at each interval over the duration of drug exposure.

2.4 Experimental design

Tissue slice superfusion has been used to investigate the effects of D-amphetamine on dopamine release at a concentration of $10 \mu\text{M}$ (Herdon et al., 1985, Parker et al., 1986). Johnson and co-workers (1986) demonstrated that MDA, MDMA and related analogues provoked 5-HT release over a similar micromolar concentration range. Dopamine release

induced by MDMA has also been investigated over this concentration range (Fitzgerald et al. 1990; Fitzgerald and Reid, 1993; Schmidt et al., 1994; Fischer et al., 2000). Riegert and co-workers in 2008 showed that MDMA (3 μ M) enhanced the spontaneous outflow of dopamine from striatal slices. Based on the above, we tested concentrations of caffeine and MDMA over a micromolar range deemed physiologically relevant for determination of drug-induced release from tissue slices under superfusion conditions. Variations between effective concentrations used between different laboratories relate to the tissue slice preparation, the perfusion system and the superfusion conditions employed. As the superfusion technique is carried out under flow conditions *ex vivo*, it does not directly represent the intact physiological condition. Consequently concentrations of drug employed are an order of magnitude higher than those required to provoke dopamine release *in vivo*.

MDMA was obtained as a gift from the National Institutes of Drug Abuse (NIDA), USA. Caffeine was obtained commercially from Sigma Alrich, Ireland.

Study 1: Dose dependent effects of MDMA and caffeine on [³H] dopamine release from striatal and hypothalamic tissue slices.

To determine the most suitable concentration of MDMA required to induce [³H] dopamine release from both striatal and hypothalamic slices, buffer containing various concentrations of MDMA (0, 30, 100 and 300 μ M) was superfused onto striatal or hypothalamic slices. Similarly the effect of caffeine (0, 10, 30 and 100 μ M) on [³H] dopamine release was also determined in striatal and hypothalamic slices.

Study 2: Effect of caffeine on MDMA-induced [³H] dopamine release from striatal and hypothalamic tissue slices

Based on the responses obtained in study 1, appropriate concentrations of caffeine and MDMA were selected and the effect of caffeine (30 μ M) on MDMA (30 or 100 μ M)-induced [³H] dopamine release from striatal and hypothalamic slices respectively was determined. As caffeine (30 μ M) did not influence [³H] dopamine release from hypothalamic tissue slices in response to the application of MDMA (30 μ M), a higher concentration of MDMA (100 μ M) was also tested in combination with caffeine.

Study 3: Effect of DPCPX, CCPA, rolipram and SCH 58261 on MDMA-induced [³H] dopamine release from striatal tissue slices

We further investigated if caffeine's ability to modulate MDMA-induced dopamine release might be simulated by application of the selective high affinity A₁ receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), the selective A_{2A} receptor antagonist, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261) or the selective phosphodiesterase inhibitor (PDE)-4 inhibitor, rolipram. PDE-4 was specifically targeted as we have previously determined its participation over other PDE isoforms in the ability of caffeine to influence the toxicity of MDMA in rats (Vanattou-Saïfoudine et al., 2010). We also exposed our striatal tissue slices with a selective adenosine A₁ agonist, 2-chloro-N(6)-cyclopentyladenosine (CCPA) to confirm a role for these receptors in caffeine's ability to enhance MDMA-induced dopamine release. All drugs were obtained from Sigma Aldrich, Ireland. At this point the experiments were restricted to the measurement of dopamine release from striatal slices as caffeine (30 μ M) failed to significantly influence MDMA (100 μ M)-induced dopamine release from hypothalamic slices. Buffer containing DPCPX (1 μ M), SCH 58261 (1 μ M), rolipram (30 μ M) or CCPA

(1 μ M) alone or in combination with MDMA (30 μ M) were superfused onto striatal slices . The concentration of DPCPX was selected from previous reports where DPCPX has been shown to be considerably more potent than caffeine at the adenosine A₁ receptor (Fredholm et al., 1999). The doses of CCPA and SCH 58261 were in line with their in vitro potencies selected based on pilot experiments in our laboratory and following review of previous reports, where these drugs were applied over a nano to micromolar concentration range in tissue slice superfusion experiments (Marchi et al., 2002). For the purpose of this study, we investigated a role for adenosine A₁ and A_{2A} receptors as caffeine mainly binds adenosine A₁ and A_{2A} receptors with high affinity compared to adenosine A_{2B} or A₃ receptors (Fisone et al. 2004). Moreover adenosine A₁ and A_{2A} receptors are most implicated in regulating dopamine transmission in the striatum.

2.5 Statistical analysis

A GB-Stat software package was used for the statistical analyses. One- or two-way ANOVA or repeated measures analysis of variance (ANOVA) followed by Dunnett's or Student Newman Keuls post-hoc comparison tests were applied to determine significant differences between the treatment groups. Results are reported as mean $\pm$ S.E.M. and differences were deemed significant at * P <0.05 or ** P <0.01

3. Results

The results show a concentration dependant increase of [^3H] dopamine release from striatal and hypothalamic tissue slices following exposure to MDMA (30, 100 and 300 μM) or caffeine (100 μM). Furthermore, caffeine (30 μM) increased MDMA (30 μM)-induced [^3H] dopamine release in the striatum while it did not influence MDMA (100 μM)-induced [^3H] dopamine release in the hypothalamus. The adenosine receptor A_1 antagonist DPCPX (1 μM) produced a caffeine-like effect on MDMA-induced dopamine release in striatal slices while SCH 58261 (1 μM) or rolipram (30 μM) failed to reproduce this effect. CCPA (1 μM) attenuated MDMA-induced [^3H] dopamine release in the striatum.

3.1 MDMA provokes [^3H] dopamine release and caffeine potentiates MDMA-induced [^3H] dopamine release from striatal tissue slices.

3.1.1 Caffeine provokes [^3H] dopamine release from striatal tissue slices.

Time course: ANOVA of [^3H] dopamine outflow showed effects of caffeine [$F(3,16) = 5.18$, $P = 0.018$], time [$F(16,256) = 28.01$, $P < 0.001$] and a caffeine x time [$F(48,256) = 2.23$, $P < 0.001$] interaction. Post hoc comparisons revealed caffeine (100 μM) induced dopamine release from striatal slices 20, 24, 28, 32 and 40 min following initial drug exposure (Fig. 1A).

AUC: ANOVA of area under the curve showed effects of caffeine on [^3H] dopamine release [$F(3, 16) = 4.56$, $P = 0.017$]. Post hoc comparisons revealed that caffeine (100 μM) increased the area under the curve when compared to vehicle treated controls (Fig. 1A, inset).

3.1.2 MDMA provokes [^3H] dopamine release from striatal tissue slices

Time course: ANOVA of [^3H] dopamine outflow showed effects of time [$F(16,288) = 12.84$, $P < 0.001$] and a MDMA x time interaction [$F(48,288) = 2.51$, $P < 0.001$]. Post hoc comparisons revealed that MDMA (300 μM) induced dopamine release 16, 20, 24, 28 and 32 min following initial drug exposure when compared to vehicle treated controls (Figure 1B).

AUC: ANOVA of area under the curve showed effects of MDMA on [^3H] dopamine release [$F(3, 18) = 3.42$, $P = 0.04$]. Post hoc comparisons revealed that MDMA (300 μM) increased the area under the curve when compared to vehicle treated controls (Fig. 1B, inset).

3.1.3 Caffeine (30 μM) potentiates MDMA (30 μM)-induced [^3H] dopamine release

Time course: ANOVA of [^3H] dopamine outflow showed effects of caffeine [$F(1,15) = 26.45$, $P < 0.001$], MDMA [$F(1, 15) = 10.03$, $P = 0.006$], caffeine x MDMA [$F(1,15) = 12.37$, $P = 0.031$], time [$F(16,240) = 22.69$, $P < 0.001$], MDMA x time [$F(16,240) = 17.07$, $P < 0.001$], caffeine x time [$F(16,240) = 9.71$, $P < 0.001$] and caffeine x MDMA x time [$F(16,240) = 10.89$, $P < 0.001$] interactions. Post hoc comparisons revealed that caffeine enhanced MDMA- induced dopamine release from striatal slices 12, 16, 20, 24, 28 and 32 min following initial drug exposure (Fig. 1C).

AUC: ANOVA of the area under the curve showed effects of MDMA [$F(1,15) = 29.27$, $P < 0.001$] only. Post hoc comparisons revealed that caffeine in combination with MDMA provoked dopamine release when compared to MDMA or caffeine treated groups (Fig. 1C, inset).

3.2 Caffeine does not influence MDMA-induced [^3H] dopamine release from hypothalamic tissue slices.

3.2.1 Caffeine provokes [^3H] dopamine release from hypothalamic tissue slices.

Time course: ANOVA of [^3H] dopamine outflow showed effects of time [$F(16,224) = 23.11$, $P < 0.001$] and caffeine x time interaction [$F(48,224) = 3.26$, $P < 0.001$]. Post hoc comparisons revealed that caffeine (100 μM)- induced dopamine release from hypothalamic tissue slices 20, 24, and 28 min following initial drug exposure when compared to vehicle treated controls (Fig. 2A).

AUC: ANOVA of area under the curve showed effects of caffeine on [^3H] dopamine release [$F(3,14) = 4.25$, $P = 0.025$]. Post hoc comparisons revealed that caffeine (100 μM) increased the area under the curve when compared to vehicle treated controls (Fig. 2A, inset).

3.2.2 MDMA provokes [^3H] dopamine release from hypothalamic tissue slices.

Time course: ANOVA of [^3H] dopamine outflow showed effects of MDMA [$F(3,14) = 33.61$, $P < 0.001$], time [$F(16,224) = 30.59$, $P < 0.001$] and MDMA x time interaction [$F(48,224) = 9.32$, $P < 0.001$]. Post hoc comparisons revealed that MDMA (100 μM)- induced dopamine release from hypothalamic tissue slices 16, 20 and 24 min following initial drug exposure when compared to vehicle treated controls. MDMA (300 μM)-induced dopamine release 12, 16, 20, 24, 28, 32 and 36 min following initial drug exposure when compared to vehicle treated controls (Fig. 2B).

AUC: ANOVA of the area under the curve showed effects of MDMA [$F(3,14) = 36.13$, $P < 0.001$]. Post hoc comparisons revealed that MDMA (100 μ M and 300 μ M) increased the area under the curve when compared to vehicle treated controls (Fig. 2B, inset).

3.2.3 Caffeine (30 μ M) does not influence MDMA (100 μ M) -induced [3 H] dopamine release from hypothalamic tissue slices.

Time course: ANOVA of [3 H] dopamine outflow showed effects of MDMA [$F(1, 15) = 50.30$, $P < 0.001$], time [$F(16, 240) = 16.84$, $P < 0.001$], MDMA x time [$F(16, 240) = 12.03$, $P < 0.001$] only. Post hoc comparisons revealed that MDMA induces dopamine release from striatal hypothalamic slices 16, 20, 24, 28 and 32 min following initial drug exposure which is not influenced by caffeine (Fig. 2C).

AUC: ANOVA of the area under the curve showed effects of MDMA [$F(1, 15) = 41.51$, $P < 0.001$] only. Post hoc comparisons revealed that MDMA provoked dopamine release when compared to vehicle treated groups. Co-administration of caffeine failed to influence the response to MDMA (Fig. 2C, inset).

3.3 DPCPX but not SCH 58261 or rolipram simulates the effects of caffeine on MDMA-induced dopamine release from striatal tissue slices.

3.3.1 DPCPX

Time course: ANOVA of [3 H] dopamine outflow from striatal slices showed effects of MDMA [$F(1,20) = 22.37$, $P = 0.0001$], DPCPX [$F(1,20) = 10.68$, $P = 0.003$], MDMA x DPCPX [$F(1,20) = 5.67$, $P = 0.027$], time [$F(16,320) = 24.21$, $P < 0.001$], MDMA x time

[F(16,320) = 10.96, $P < 0.001$], DPCPX x time [F(16,320) = 6.48, $P < 0.001$] and MDMA x DPCPX x time interaction [F(16,320) = 4.27, $P < 0.001$]. Post hoc comparisons revealed that co-administration of DPCPX with MDMA provoked dopamine release when compared to DPCPX or MDMA treatments alone 16, 20, 24, 28 and 32 min following initial drug exposure (Fig. 3A).

AUC: ANOVA of the area under the curve showed effects of DPCPX [F(1, 20) = 11.05, $P = 0.0034$], MDMA [F(1, 20) = 17.64, $P = 0.0004$] and DPCPX x MDMA interaction [F(1, 20) = 5.77, $P = 0.026$]. Post hoc comparisons revealed that co-administration of DPCPX with MDMA provoked dopamine release when compared to DPCPX or MDMA treatments alone (Fig. 3A, inset).

3.3.2 CCPA

Time course: ANOVA of [^3H] dopamine outflow from striatal slices showed effects of MDMA [F(1,16) = 4.36, $P = 0.05$], time [F(16,256) = 5.52, $P < 0.001$], MDMA x time [F(16,256) = 2.36, $P = 0.003$], CCPA x time [F(16,256) = 2.24, $P = 0.005$] and MDMA x CCPA x time interactions [F(16,256) = 1.94, $P = 0.016$]. Post hoc comparisons revealed that MDMA induced [^3H] dopamine release 28, 32, 36 and 40 min following drug exposure when compared to vehicle treated controls. CCPA alone did not influence dopamine release but it attenuated MDMA-induced dopamine release 28, 32, 36 and 40 min when compared to MDMA treatment alone (Fig. 3B).

AUC: ANOVA of the area under the curve showed effects of CCPA [F(1,16) = 5.16, $P = 0.04$], MDMA [F(1,16) = 5.57, $P = 0.03$] and a MDMA x CCPA interaction [F(1,16) = 7.29, $P = 0.016$]. Post hoc comparisons revealed that MDMA increased dopamine release when

compared to vehicle treated controls. CCPA had no effect on dopamine release when compared to vehicle treated controls. Co-administration of CCPA attenuated MDMA-induced dopamine release when compared to MDMA treatment alone (Fig. 3B, inset).

3.3.3 SCH 58261

Time course: ANOVA of [^3H] dopamine outflow from striatal slices showed effects of MDMA [$F(1,18) = 11$, $P = 0.038$], time [$F(16,288) = 10.31$, $P < 0.001$], MDMA x time [$F(16,288) = 5.22$, $P < 0.001$] and SCH 58261 x time [$F(16,288) = 2.49$, $P = 0.0013$] only. Post hoc comparisons revealed MDMA induced [^3H] dopamine release 28, 32, 36 and 40 min following initial drug exposure when compared to vehicle treated controls. SCH 58261 alone or in combination with MDMA did not influence dopamine release (Fig. 3C).

AUC: ANOVA of the area under the curve showed effects of MDMA [$F(1,18) = 15.39$, $P = 0.01$] only. Post hoc comparisons revealed that neither MDMA nor SCH 58261 alone or in combination influence dopamine release when compared to vehicle treated controls (Fig. 3C, inset).

3.3.4 Rolipram

Time course: ANOVA of [^3H] dopamine outflow from striatal slices showed effects of MDMA [$F(1,12) = 9.55$, $P = 0.009$], time [$F(16,192) = 8.831$, $P < 0.001$], rolipram x time [$F(16,192) = 2.07$, $P = 0.01$], MDMA x time [$F(16,192) = 4.24$, $P < 0.001$] and, MDMA x rolipram x time interactions [$F(16,256) = 5.04$, $P < 0.001$]. Post hoc comparisons revealed that MDMA induced dopamine release from striatal slices 28, 32, 36 and 40 min following initial drug exposure when compared to vehicle treated controls. Rolipram attenuated this

release 36 min after the onset of drug exposure when compared to MDMA treatment alone (Fig. 3D).

AUC: ANOVA of the area under the curve showed effects of MDMA [$F(1,12) = 11.8$, $P = 0.0049$] only. Post hoc comparisons revealed that MDMA increased the area under the curve which was not affected by co-treatment with rolipram (Fig. 3D, inset).

4. Discussion

The current data demonstrate that caffeine can influence MDMA-induced dopamine release from striatal but not from hypothalamic tissue slices. Higher concentrations of MDMA were required to provoke dopamine release from the hypothalamus. Thus, in order to determine if caffeine might interact with MDMA-induced dopamine release in hypothalamic slices, a higher concentration of MDMA (100 μ M) was tested when compared to slices prepared from the striatum. When tested in combination, caffeine (30 μ M) enhanced MDMA (30 μ M)-induced dopamine release from striatal tissue slices. By contrast, caffeine (30 μ M) did not influence MDMA (100 μ M)-induced dopamine release from hypothalamic slices. As caffeine influenced MDMA-induced [3 H] dopamine release from striatal slices, further work was focused on elucidating the mechanism by which caffeine produces this response. As caffeine shows a preferential affinity for adenosine A₁ receptors (Ferre et al., 2008; Nehlig, 1999; Fredholm et al., 1999a), additional experiments were carried out to determine if adenosine A₁ receptors might be implicated in the ability of caffeine to influence MDMA-induced dopamine release and striatal slices were exposed to DPCPX or CCPA alone and in combination with MDMA. Application of DPCPX produced a caffeine-like action by provoking an increase in dopamine release following exposure to MDMA. In support of an adenosine A₁ receptor mechanism mediating the effects of DPCPX, co-treatment of MDMA with the adenosine A₁ receptor agonist CCPA induced an opposite effect to that observed with DPCPX. As caffeine is also known to act as an antagonist of adenosine A_{2A} receptors (Fredholm et al., 1999a; Nehlig, 1999) and is also a weak PDE inhibitor (Fredholm and Lindström, 1999), we further investigated the role of these potential targets in caffeine effects on MDMA induced dopamine release. SCH 58261, the adenosine A_{2A} receptor antagonist failed to produce a caffeine-like effect suggesting that this target is not implicated in the

actions of caffeine. A high concentration of the PDE-4 inhibitor rolipram (30 μ M) was required to induce a reduction in MDMA induced dopamine release. This effect was opposite to that observed with caffeine. It is therefore reasonable to conclude that the PDE inhibitory properties of caffeine are unlikely to contribute to its ability to enhance MDMA-induced dopamine release from striatal slices. Based on these findings we propose that caffeine can effect an adenosine A₁ receptor dependent regulation of MDMA-induced dopamine release in the striatum.

Several previous reports have described MDMA-induced dopamine release in superfused tissue slices, synaptosomal preparations (Schmidt et al, 1987; Johnson et al., 1986; Fitzgerald et al., 1990, 1993; Riegert et al., 2008; Johnson et al., 1991; Steele et al., 1987) and in studies using *in vivo* intracranial microdialysis (Gough et al., 1991; Nash and Nichols, 1991; Sabol and Seiden, 1998; Esteban et al., 2001; Baumann et al., 2008; Benamar et al., 2008). Several investigations have also been carried out on the effects of caffeine (Borycz et al., 2007, Bonanno et al., 2000) on dopamine release from superfused tissue slices. These studies were performed on striatal, accumbal and hippocampal tissues but not on hypothalamic tissue. Earlier experiments too have shown that caffeine increases dopamine and glutamate release in the striatum via the blockade of inhibitory pre-synaptic adenosine A₁ receptors (Okada et al., 1996, Solinas et al.; 2002, Quarta et al., 2004; Ciruela et al., 2006; Borycz et al., 2007). Such a mechanism is consistent with the observations of the present study where caffeine enhances MDMA-induced dopamine release in the striatum through an adenosine A₁ receptor mechanism. The effects of caffeine and DPCPX on MDMA-induced dopamine release in the striatum may generalise to other amphetamines as there are reports of adenosinergic modulation of methamphetamine-induced striatal dopamine release (Golembiowska and

Zylewska, 1998) and methamphetamine-induced sensitization to striatal dopamine release (Yoshimatsu et al., 2001; Shimazoe et al., 2000).

The differential response obtained with caffeine on MDMA-induced dopamine release in hypothalamic by comparison to striatal tissue slices suggests that the effects of caffeine are regionally dependent. Dopaminergic afferents and receptors in the hypothalamus and their neuroanatomical and neurochemical links with the regulation of body temperature, sleep regulation and sexual behaviour have been reviewed elsewhere (Kelley et al., 2002, Kumar et al., 2007, Dominguez et al., 2005). Although adenosine receptors are distributed in many brain regions including the hypothalamus (Ferre et al., 2007; Ochiishi et al., 1999), their pre-synaptic regulation of dopamine in addition to GABA and glutamate release, as described in the striatum, has not been reported in hypothalamic nuclei to date.

By contrast to adenosine A₁ receptors, stimulation of adenosine A_{2A} receptors increases extracellular concentrations of dopamine and glutamate in the striatum (Popoli et al., 1995; Okada et al., 1996; Golembiowska and Zylewska, 1997). Opposite modulatory roles for adenosine A₁ and A_{2A} receptors on glutamate and dopamine release have been described in the shell of the nucleus accumbens (Quarta et al., 2004). In addition adenosine A₁ receptors are known to inhibit adenosine A_{2A} receptors (Quarta et al., 2004; Okada et al., 1996; Karcz-Kubicha et al., 2003). As such interactions may also be of relevance to the mechanisms mediating the ability of caffeine to promote MDMA-induced dopamine release, the effects of the selective adenosine A_{2A} antagonist SCH 58261 were also examined. By contrast to DPCPX and CCPA however, SCH 58261 failed to influence dopamine release from striatal slices either alone or in combination with MDMA. The lack of a response with SCH 58261

suggests that adenosine A_{2A} receptors do not play a role in mediating the ability of caffeine to influence MDMA-induced dopamine release.

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 availability following MDMA-induced biogenic amine release in the brain. 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., 1992). Similarly, we have recently reported that the PDE inhibitory properties of caffeine in part contribute to the mechanisms mediating the ability of caffeine to influence MDMA-induced hyperthermia in rats (Vanattou-Saïfoudine et al., 2010). In the present investigation however, similar to the results obtained with SCH 58261, co-treatment with the PDE inhibitor rolipram failed to provoke a caffeine-like interaction with MDMA, indicating that PDE inhibition fails to account for the interaction observed between caffeine and MDMA in the present investigation.

As caffeine provokes a potentially lethal interaction with MDMA *in vivo*, the question arises as to the extent to which dopamine release may contribute to the toxicity. The present series of experiments determine the influence of drugs on the spontaneous release of dopamine from superfused tissue slices. Experiments employing electrical field stimulation (EFS) evoked transmitter release, in attempts to more closely simulate the *in vivo* condition, would also be of interest although such experiments are not described here. Others who have used

EFS have previously reported that MDMA can reduce electrically evoked dopamine release from striatal slices whereas in the same series of experiments MDMA enhanced the spontaneous outflow of dopamine (Riegert et al., 2008). Thus EFS can provoke marked differences with regard to drug response and such differences warrant consideration and further experimentation. Our work to date has largely addressed mechanisms mediating the ability of caffeine to exacerbate MDMA-induced hyperthermia and in this regard a role for dopamine has been described (McNamara et al., 2006; Vanattou-Saïfoudine et al., 2010a,b). We have reported previously that depletion of endogenous catecholamines in the hypothalamus or pre-treatment with the dopamine D₁ receptor antagonist SCH 23390 attenuates the ability of caffeine to exacerbate MDMA-induced hyperthermia (Vanattou-Saïfoudine et al., 2010a). We have also determined that caffeine can potentiate MDMA-induced behavioural responses including locomotor activity and 5-HT syndrome. One mechanism by which caffeine may potentiate dopamine-mediated changes in core body temperature or behaviour is through a potentiation of MDMA-induced dopamine release. Although such a mechanism may be relevance in the striatum and striatal mediated behaviours, caffeine in fact did not influence MDMA-induced dopamine release in hypothalamic tissue slices. Thus pre-synaptic dopamine release in the hypothalamus is unlikely to account for the ability of caffeine to exacerbate MDMA-induced hyperthermia. Alternatively post-synaptic adenosinergic and dopaminergic mechanisms will need to be examined in order to account for the ability of caffeine to enhance MDMA-induced hyperthermia.

In conclusion, the results of this study show that caffeine differentially regulates MDMA-induced dopamine release from striatal tissue slices and this effect is most likely mediated by adenosine A₁ receptor blockade. Whilst the ability of caffeine to enhance MDMA-induced

dopamine release from striatal slices may play a role in toxicity observed *in vivo*, post-synaptic mechanisms should also be investigated to clarify the mechanisms by which caffeine exacerbates MDMA-induced toxicity.

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Figure legends

Fig. 1: MDMA dose- dependently induces [³H]dopamine release and caffeine potentiates MDMA-induced [³H]dopamine release from striatal slices.

There was no difference in [³H] dopamine outflow between the groups prior to drug exposure. Values represent mean with standard error of the mean. The period of drug exposure to tissue slices is indicated by the bold line. The area under the curve (AUC) for the period of time tissue slices were exposed to the drugs is also shown in the inset. *P < 0.05, **P < 0.01 vs. Vehicle Control; +P < 0.01 vs. Vehicle + MDMA. **(A)** Caffeine (100μM) induces [³H] dopamine release: N = 5 animals per group. **(B)** MDMA (300μM) induces [³H] dopamine release: N = 6 animals per group. **(C)** Caffeine (30μM) potentiates MDMA (30μM)-induced [³H] dopamine release N = 4-5 animals per group.

Fig. 2: MDMA induced [³H] dopamine release from hypothalamic slices is not influenced by caffeine.

There was no difference in [³H] dopamine outflow between the groups prior to drug exposure. Values represent mean with standard error of the mean. The period of drug exposure to tissue slices is indicated by the bold line. The area under the curve (AUC) for the period of time tissue slices were exposed to the drugs is also shown in the inset. *P < 0.01 vs. Vehicle Control **(A)** Caffeine (100μM) induces [³H] dopamine release: N = 4-5 animals per group. **(B)** MDMA (100 and 300μM) induces [³H] dopamine release: N = 4-5 animals per group. **(C)** Caffeine (30μM) did not influence MDMA (100μM)-induced [³H] dopamine release: N = 4-5 animals per group.

Fig. 3: DPCPX but not SCH 58261 or rolipram simulates the effects of caffeine on MDMA-induced dopamine release from striatal tissue slices

There was no difference in [^3H] dopamine outflow between the groups prior to drug exposure. Values represent mean with standard error of the mean. . The period of drug exposure to tissue slices is indicated by the bold line. The area under the curve (AUC) for the period of time tissue slices were exposed to the drugs is also shown in the inset. * $P < 0.01$ vs. Vehicle Control; + $P < 0.01$ vs. Vehicle + MDMA. **(A)** DPCPX (1 μM) potentiates MDMA (30 μM)-induced [^3H] dopamine release: $N = 6$ animals per group. **(B)** CCPA (1 μM) attenuates MDMA (30 μM)-induced [^3H] dopamine release: $N = 5$ animals per group. **(C)** SCH 58261 (1 μM) fails to produce a caffeine-like effect on MDMA (30 μM)-induced [^3H] dopamine release: $N=5-6$ animals per group. **(D)** Rolipram (30 μM) fails to produce a caffeine-like on MDMA (30 μM)-induced [^3H] dopamine release. $N = 4-5$ animals per group

Figure 1

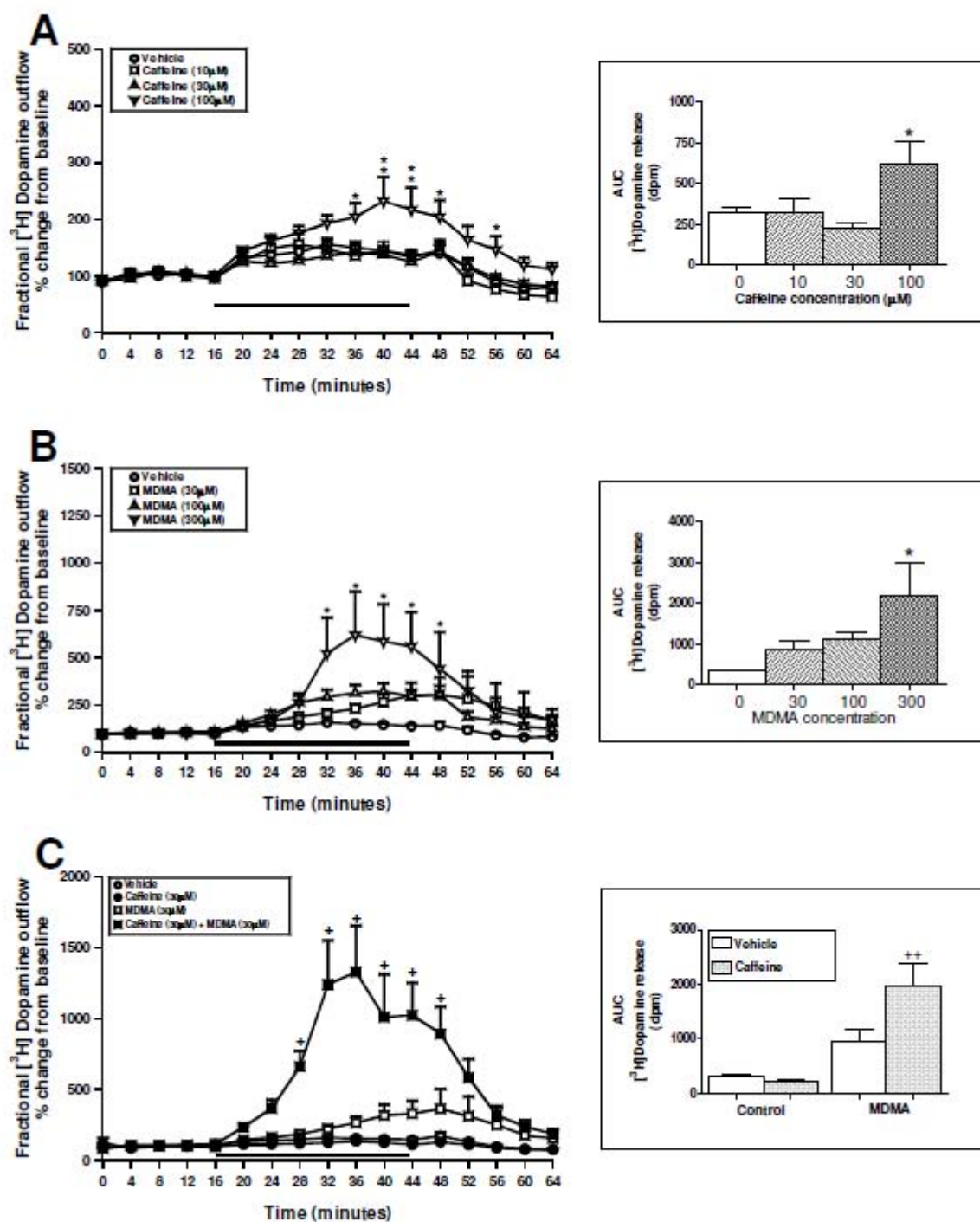


Figure 2

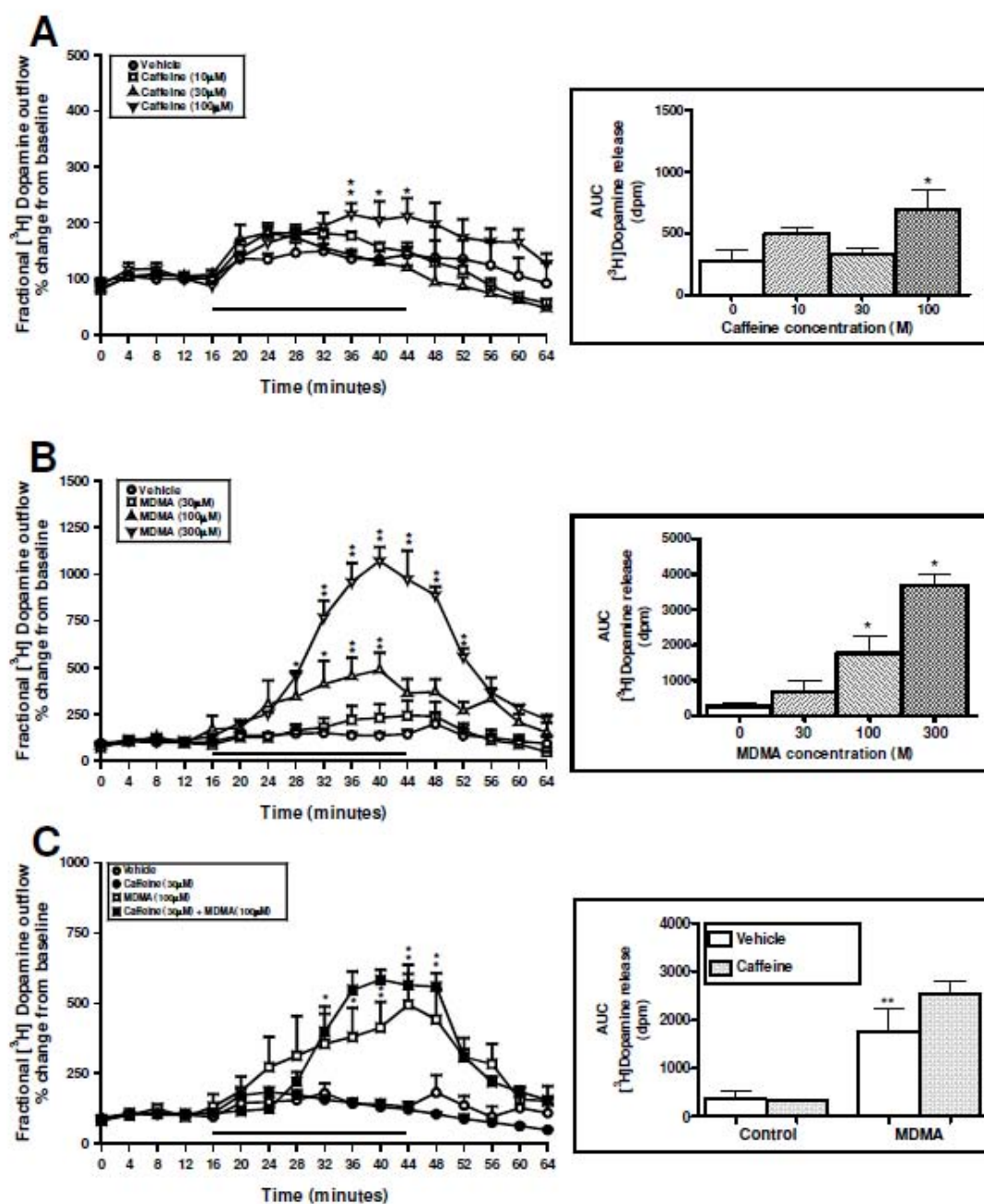


Figure 3

