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REVIEW

# Caffeine provokes adverse interactions with 3,4-methylenedioxy-methamphetamine (MDMA, 'ecstasy') and related psychostimulants: mechanisms and mediators

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Concomitant consumption of caffeine with recreational psychostimulant drugs of abuse can provoke severe acute adverse reactions in addition to longer term consequences. The mechanisms by which caffeine increases the toxicity of psychostimulants include changes in body temperature regulation, cardiotoxicity and lowering of the seizure threshold. Caffeine also influences the stimulatory, discriminative and reinforcing effects of psychostimulant drugs. In this review, we consider our current understanding of such caffeine-related drug interactions, placing a particular emphasis on an adverse interaction between caffeine and the substituted amphetamine, 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'), which has been most recently described and characterized. Co-administration of caffeine profoundly enhances the acute toxicity of MDMA in rats, as manifested by high core body temperature, tachycardia and increased mortality. In addition, co-administration of caffeine enhances the long-term serotonergic neurotoxicity induced by MDMA. Observations to date support an interactive model of drug-induced toxicity comprising MDMA-related enhancement of dopamine release coupled to a caffeine-mediated antagonism of adenosine receptors in addition to inhibition of PDE. These experiments are reviewed together with reports of caffeine-related drug interactions with cocaine, d-amphetamine and ephedrine where similar mechanisms are implicated. Understanding the underlying mechanisms will guide appropriate intervention strategies for the management of severe reactions and potential for increased drug-related toxicity, resulting from concomitant caffeine consumption.

### Abbreviations

BAT, brown adipose tissue; BP, blood pressure; CPP, conditioned place preference; CREB, cAMP response element binding protein; CVS, cardiovascular system; CYP, cytochrome P450 enzyme; DARPP-32, cAMP-regulated phosphoprotein of 32 kDa; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; MDA, methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; PDE, phosphodiesterase; UCP, uncoupling protein

## Introduction

In recreational drug use settings, caffeine is found in a range of commercially available products such as energy drinks that may be consumed with other drugs (Reissig *et al.*, 2009). In addition, forensic analyses of seized illicit drug samples have reported quantities of caffeine mixed with other stimulants, including cocaine and amphetamines (Cole *et al.*, 2011).

Such combinations may influence the toxicity of these stimulants and, consequently, investigations regarding the potential for adverse interactive effects are warranted. Interactive effects have led to concern that caffeine could have a role to play in the acute, often idiosyncratic, and long-term adverse consequences associated with the consumption of a range of psychostimulant drugs, including some agents in clinical use.

## Risks associated with caffeine use in recreational drug users

Reviews of the toxicity of caffeine and detailed accounts of presentations of caffeine excess and overdose including nervousness, agitation, anxiety and insomnia are found elsewhere (Abbott, 1986). More than 300 million European Union citizens consume caffeine on a daily basis, with daily intake ranging from 50 to 150 mg. Fatalities related to caffeine overdose are rare and associated with oral doses of between 3 and 20 g (Holmgren *et al.*, 2004).

Although habitual caffeine consumption is generally regarded as safe, caffeine has the potential to influence the toxicity of other stimulants in a profound way. One of the first reports to signal this potential was a series of experiments performed in rats where administration of caffeine with either amphetamine or cocaine resulted in seizures and mortality when compared with administration of dextroamphetamine (d-amphetamine) or cocaine alone (Derlet *et al.*, 1992). More recently, further preclinical data demonstrated that caffeine profoundly increases the acute toxicity of the substituted amphetamines 3,4-methylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA) in the rat, as characterized by seizures, hyperthermia, tachycardia and lethality, which are not observed following administration of equivalent doses of MDMA or MDA alone (Camarasa *et al.*, 2006; McNamara *et al.*, 2006; 2007). In addition, caffeine was shown to promote the long-term loss of central serotonin (5-HT), which is commonly reported following substituted amphetamine administration to rodents and proposed as a marker of the long-term degeneration of 5-HT neuronal systems associated with repeated administration of these drugs (McNamara *et al.*, 2006).

From reports to date, it appears that higher doses of caffeine (100 mg) are required to promote the toxicity of d-amphetamine and cocaine as reported by Derlet *et al.* (1992), while lower doses of caffeine (5–20 mg) are sufficient to promote toxicity and lethality when combined with MDMA or MDA (McNamara *et al.*, 2006). Interactions between caffeine and d-amphetamine or cocaine have not been observed over the same dose range of caffeine (i.e. 5–20 mg·kg$^{-1}$), where profound interactions were observed with MDMA. Thus, the substituted amphetamines appear particularly sensitive to the toxicity-augmenting effects of caffeine. Mechanisms contributing to this sensitivity are likely to relate to the dual action of MDMA on both 5-HT and dopaminergic neuronal systems. In support of this view, co-administration of caffeine (5–20 mg·kg$^{-1}$) with either the selective 5-HT releasing drug d-fenfluramine or d-amphetamine, which primarily stimulates the release of catecholamines, is insufficient to promote toxicity leading to fatality. By contrast, co-administration of caffeine with a combination of d-fenfluramine and d-amphetamine provokes a severe toxic interaction similar to that seen following caffeine and MDMA co-administration (Vanattou-Saïfoudine *et al.*, 2010a) (Table 1).

Clinical studies of the interaction between caffeine and other stimulants are largely focussed on the influence of caffeine on their abuse liability. There are few reports that are specifically relevant to the acute toxicity and safety of these interactions in humans and relatively little research has been primarily aimed at testing the acute interactive effects of caffeine in drug users. If toxicity outcomes such as those that have been reported in laboratory animals to date translate to humans, the interaction between caffeine and MDMA may place polydrug abusers in danger of severe toxic reactions with potential for loss of life. Ecstasy use has been associated with deaths and dangerous adverse effects, with multiple lines of evidence suggesting that it can be neurotoxic. Hyperthermia is a primary feature of MDMA-induced toxicity in humans and is the leading cause of emergency-room admissions following problems induced by this drug. However, the majority of admissions are unrelated to the dose of drug consumed and blood levels of these drugs cannot be used to predict toxicity (Burgess *et al.*, 2000), leading to speculation that either deliberate or inadvertent co-consumption of other stimulant drugs may contribute to the adverse reactions observed. In this regard, caffeine is commonly reported as an adulterant in cocaine and amphetamine samples obtained on the streets or from law enforcement drug seizures (Cole *et al.*, 2011). Not only has caffeine been found to be present in as many as 20% of ecstasy tablets analysed, but caffeinated 'energy drinks' have gained widespread popularity within nightclub or rave environments where ecstasy is frequently consumed. The consumption of such products alone may pose a risk for some vulnerable individuals developing adverse effects, including anxiety symptoms and heart palpitations (Reissig *et al.*, 2009). It is therefore conceivable that such products would serve to promote and exacerbate adverse reactions when taken in combination with more potent illicit psychostimulants.

Ethanol and caffeine are frequently taken in combination through mixing alcoholic and caffeinated beverages. Reasons for concern related to energy drink consumption in combination with alcohol arise from the potential for adverse effects as concurrent caffeine consumption may contribute to underestimated levels of alcohol-related impairment, leading to serious alcohol-related consequences and a higher risk for alcohol dependence (Arria and O'Brien, 2011). Caffeine may offset some of the intoxicating effects of alcohol, which may serve to increase alcohol intake in some drug users. In a study of social drinkers, Marczinski and Fillmore (2006) reported that co-administration of caffeine with alcohol reduced participants' perceptions of alcohol intoxication compared with administration of alcohol alone. Further studies are required to more fully characterize the potential dangers of combining high-energy caffeine drinks with ethanol. In the interim, it appears that commercially available caffeinated beverages are likely to come under stricter regulations and controls (Nature Editorial, 2010; Benac, 2011).

The Food and Drug Administration and other regulatory agencies have warned against the combination of caffeine

## Table 1

Summary of the reported caffeine interactions with MDMA and related psychostimulants

| Caffeine | Psychostimulant | Nature of interaction | Reference |
|---|---|---|---|
| 20 mg·kg$^{-1}$, i.p., once daily × 9 days | Cocaine 10 mg·kg$^{-1}$, i.p. | Increased locomotor effects in rats | Schenk *et al.*, 1990 |
| 10 mg·kg$^{-1}$, i.p. | Cocaine 1–17.5 mg·kg$^{-1}$, i.p.<br>d-amphetamine 0.1–1 mg·kg$^{-1}$, i.p. | Potentiation of discriminative stimulus effects in rats | Mumford and Holtzman, 1991 |
| 100 mg·kg$^{-1}$, i.p. | Cocaine 0–90 mg·kg$^{-1}$, i.p.<br>d-amphetamine 0–42 mg·kg$^{-1}$, i.p. | Increased seizures and lethality in rats<br>Increased seizures and lethality in rats | Derlet *et al.*, 1992 |
| In drinking water 3 mg·mL$^{-1}$ | Cocaine 1–17 mg·kg$^{-1,}$ i.p.<br>d-amphetamine 0.1–5.6 mg·kg$^{-1}$, i.p. | Sensitization in a fixed-interval schedule of reinforcement in rats | Jaszyna *et al.* 1998 |
| 1.25–20 mg·kg$^{-1}$, i.p. | Cocaine 0.5 mg·kg$^{-1}$ per infusion | Increased cocaine self-administration in rats | Schenk and Partridge, 1999 |
| 20 mg·kg$^{-1}$ twice daily × 4 days | MDMA 20 mg·kg$^{-1}$ twice daily × 4 days | Increased lethality, hyperthermia and decreased cortical serotonin transporter density in rats | Camarasa *et al.*, 2006 |
| 10 mg·kg$^{-1}$, s.c. | MDMA 5 mg·kg$^{-1}$, s.c. | Potentiation of spontaneous locomotor activity in mice | Camarasa *et al.*, 2006 |
| 5–20 mg·kg$^{-1}$, i.p. | MDMA 10–30 mg·kg$^{-1}$, i.p. | Increased lethality and hyperthermia in rats | McNamara *et al.*, 2006 |
| 5–20 mg·kg$^{-1}$, i.p. | MDA 5–12.5 mg·kg$^{-1}$, i.p. | Increased lethality, seizures and hyperthermia in rats | McNamara *et al.*, 2006 |
| 10 mg·kg$^{-1}$ b.i.d. × 4 days | MDA 5 mg·kg$^{-1}$ twice daily × 4 days | Long-term cortical serotonin loss in rats | McNamara *et al.*, 2006 |
| 10 mg·kg$^{-1}$ × four times daily for 4 days | MDMA 2.5 mg·kg$^{-1}$ four times daily for 2 days | Long-term hippocampal serotonin loss in rats | McNamara *et al.*, 2006 |
| 10 mg·kg$^{-1}$, s.c. | MDMA 10 mg·kg$^{-1}$, s.c. | Tachycardia in rats | McNamara *et al.*, 2007 |
| 10 mg·kg$^{-1}$, i.p. | MDMA 15 mg·kg$^{-1}$, i.p. | Hyperthermia in rats | Vanattou-Saifoudine *et al.*, 2010a |
| 10 mg·kg$^{-1}$, i.p. | d-amphetamine 1 mg·kg$^{-1}$ with d-fenfluramine 5 mg·kg$^{-1}$, i.p. | Hyperthermia in rats | Vanattou-Saifoudine *et al.*, 2010a |

Summary of the reported interactions, including details of drug doses, associated with the co-administration of caffeine with other psychostimulants in rodents.

with a range of drugs in clinical use, including low-dose methamphetamine, d-amphetamine, ephedrine and methylphenidate on the understanding that combination with caffeine could promote cardiovascular and CNS excitatory effects. Students are especially known to use stimulants, such as methylphenidate and d-amphetamine, to increase attention span in preparation for examinations and such drugs may be consumed in combination with caffeine. Student populations might therefore be considered at risk for potential interactive toxicity associated with caffeine, although such cohorts have not been specifically identified or described in the literature to date. A further case in point is the phenylaminoketone bupropion, a drug that was originally developed as an atypical antidepressant agent, which is now also widely used to aid smoking cessation (Roddy, 2004). Buproprion has a number of side effects, including convulsions and seizures (an estimated incidence of 1 in 1000 users), and has been contraindicated in patients with a history of epilepsy. A number of fatalities were reported following its release in the market, some of which were attributed to intake with caffeine or its metabolites. In addition, there have been several serious warnings regarding the dangers of inadvertent drug–drug interactions with caffeine, in light of its ubiquitous consumption and presence in many over-the-counter medications (Donovan and DeVane, 2001). As caffeine is primarily metabolized by the cytochrome enzyme CYP 1A2, which is also a major target of a wide range of psychiatric medications including antidepressant agents, antipsychotic agents, mood stabilizers, anti-anxiety and sedative agents, the possibility that caffeine may result in potentially toxic levels of non-metabolized drug in plasma and tissue is also worthy of consideration (Donovan and DeVane, 2001).

A combination of caffeine and ephedrine is becoming ever more popular as an ergogenic and performance-enhancing aid in sport, despite limited knowledge of its efficacy and safety. Caffeine and ephedrine in combination have metabolic and physiological effects during exercise, including increasing blood glucose, increasing lactate and epinephrine concentrations, enhancing pulmonary gas exchange and raising heart rate. Users also report a reduction in the rating of perceived exertion independent of the activity performed. The use of caffeine alone or in combination with other

stimulants for performance enhancement in sport has been reviewed elsewhere (for review, see Magkos and Kavouras, 2004).

## Experimental evidence for caffeine-induced increase in the toxicity of other psychostimulant drugs of abuse

### *Core body temperature changes*

Hyperthermia is a major feature of MDMA-induced toxicity where body temperatures as high as 43°C have been reported. This can lead to other complications such as rhabdomyolysis, disseminated intravascular coagulation and acute renal failure (Green *et al.*, 2003), although such severe adverse effects and related fatalities occur in a very small proportion of users. As a result, a particular emphasis has been placed in animal studies carried out to date on the characterization of the body temperature response to caffeine and MDMA alone and in combination, and elucidation of the mechanisms underlying the ability of caffeine to exacerbate MDMA-induced hyperthermia. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (McGrath *et al.*, 2010).

In this regard, it has been reported that caffeine can profoundly alter the core body temperature response to MDMA in rats at doses which do not themselves affect body temperature (McNamara *et al.*, 2006; Vanattou-Saïfoudine *et al.*, 2010a). Consistent with previous reports (Green *et al.*, 2004), MDMA (15 mg·kg$^{-1}$) provokes a 1.5–2°C rise in body temperature in group-housed rats, which persists for 3 h following drug administration. Co-administration of caffeine (1–20 mg·kg$^{-1}$) provokes a dose-dependent increase in the peak temperature response and duration of the hyperthermic response to both MDMA and MDA. Animals show qualitatively different responses to MDMA depending on the housing condition: a decrease in body temperature when housed individually or in a low ambient temperature (15°C) and an elevation in body temperature when housed in groups at standard ambient temperatures. As aggregation toxicity in rodents (defined as an increase in toxicity when subjects are housed together instead of being housed individually) has been observed for many drugs including amphetamines and caffeine, the impact of individual housing was examined as a strategy to reduce the toxicity associated with caffeine and MDMA co-administration. Co-administration of caffeine attenuates the hypothermic response to MDMA in individually housed animals and provoked a switch to hyperthermia (McNamara *et al.*, 2006).

There are several ways in which caffeine could interact with MDMA to provoke a hyperthermic response. As caffeine is a well-known inhibitor of CYP 1A2 (Kot and Daniel, 2008) and *N*-demethylation of MDMA to MDA may be catalysed in rats and humans by CYP 1A2 (Maurer *et al.*, 2000), caffeine could influence the metabolism of MDMA, resulting in a higher than usual plasma concentration of MDMA. From rodent studies, caffeine does not, however, modify bioavailable concentrations of MDMA or MDA in the brain (Vanattou-Saïfoudine *et al.*, 2010a). Consequently, a pharmacodynamic interaction is considered to be the more likely mechanism allowing caffeine to exacerbate the acute toxicity of MDMA.

MDMA stimulates the release of 5-HT and dopamine in several regions of the brain (see Green *et al.*, 2003; Gudelsky and Yamamoto, 2008). However, depletion of endogenous 5-HT does not alter the hyperthermic response to concurrent caffeine and MDMA administration to rats. In addition, co-administration of caffeine with the substituted amphetamine d-fenfluramine to rats, unlike MDMA, fails to provoke a hyperthermic response. As d-fenfluramine potently stimulates the release of 5-HT but lacks the catecholaminergic-releasing properties of MDMA and MDA, such results do not support a role for 5-HT alone in mediating the ability of caffeine to exacerbate MDMA-induced hyperthermia (Vanattou-Saïfoudine *et al.*, 2010a). As previously mentioned, however, it is also noteworthy that co-administration of caffeine (albeit at higher doses) with d-amphetamine or cocaine leads to a dramatic increase in seizures and mortality in rats in comparison with administration of d-amphetamine or cocaine alone (Derlet *et al.*, 1992). As amphetamine and cocaine increase extracellular dopamine levels, such interactions suggest a role for dopamine in mediating severe adverse reactions associated with the concurrent use of caffeine. In support of this, Vanattou-Saïfoudine *et al.* (2010a) showed that catecholamine depletion can block the hyperthermia associated with the co-administration of caffeine with MDMA to rats. The likely mechanism would therefore appear to be catecholaminergic but with a caveat. Although d-amphetamine increases core body temperature in rats by catecholamine-dependent mechanisms (Jaehne *et al.*, 2005), caffeine fails to increase d-amphetamine-induced hyperthermia and provokes an MDMA-like hyperthermic interaction only when d-amphetamine is co-administered with d-fenfluramine (Vanattou-Saïfoudine *et al.*, 2010a). These pharmacological manipulations therefore indicate that 5-HT contributes in part, and likely in concert with catecholamines, to the interaction between caffeine and MDMA.

Hyperthermia is known to play a significant role in determining the severity of the subsequent 5-HT loss associated with substituted amphetamine administration (see Green *et al.*, 2004). Thus, the ability of caffeine to exacerbate MDMA-induced hyperthermia might well contribute to the enhanced 5-HT toxicity associated with the drug combination (McNamara *et al.*, 2006). Camarasa *et al.* (2006) speculated that increased dopamine release induced by the combination of caffeine with MDMA enhances the availability of dopamine for transport into depleted 5-HT terminals where dopamine can be deaminated by MAO oxidative products that selectively destroy the 5-HT terminal. Alternative or additional mechanisms to hyperthermia associated with the ability of caffeine to increase MDMA-induced long-term 5-HT loss are also possible such as increased oxidative and metabolic stress, but these remain to be more fully elucidated.

### *Cardiovascular toxicity*

Several clinical studies suggest a cardiovascular interaction between caffeine and other psychostimulant drugs of abuse. Cocaine is a powerful cardiostimulant that, as a result of vasoconstriction of the coronary arteries, can induce myocardial infarction and arrhythmia. Furthermore, it can also produce hypertension and tachycardia (Phillips *et al.*, 2009).

As caffeine is also known to promote an acute rise in blood pressure (BP) (Riksen *et al.*, 2009), combined intake with cocaine is a cause for concern with regard to the induction of a potentially lethal cardiovascular event in vulnerable individuals. Although the effects of cocaine and caffeine alone are documented, to date there are few reports of the combined effects of both drugs on cardiovascular function. Mehta *et al.* (2004) confirmed in a canine model that caffeine and cocaine generated synergistic excitatory effects on the CVS. More recently in a clinical study, acute oral caffeine was reported to augment the BP response to i.v. cocaine (Johnson *et al.*, 2010).

Acute cardiovascular effects associated with ecstasy use include elevations in BP and heart rate (Dumont and Verkes, 2006), which may lead to adverse events such as hypertensive emergencies, arrhythmias and cardiotoxicity, when high quantities or repeated doses of the drug are consumed (Badon *et al.*, 2002; Green *et al.*, 2003). In our investigations to date, in addition to the ability of caffeine to promote hyperthermia when co-administered with MDMA, a profound and sustained tachycardic response is evident in rats compared with animals treated with either drug alone (McNamara *et al.*, 2007; Vanattou-Saïfoudine *et al.*, 2010b). As some fatalities occur within 15–30 min of drug administration, a time at which a significant degree of hyperthermia is not evident, our data argue against a predominant role for hyperthermia in predicting lethality. It is therefore conceivable that the ability of caffeine to induce lethality in response to MDMA is mediated by a cardiac event that may also be a contributing factor in MDMA-related deaths in humans.

As neither caffeine nor MDMA alone, or in combination, affected the electrocardiogram of the isolated heart, it has been suggested that central and sympathomimetic actions, rather than direct actions of these drugs on the heart, are responsible for the tachycardia observed *in vivo* (McNamara *et al.*, 2007). Clinically, MDMA has acute cardiovascular effects, including a dose-dependent chronotropic effect on the heart, with low doses inducing a moderate increase in heart rate and higher doses inducing bradycardia. Pressor-mediated baroreflex activation and vagal nerve stimulation have been proposed as the mechanisms responsible for MDMA-induced bradycardia. Co-administration of caffeine with MDMA may override these mechanisms, inducing a switch to tachycardia as previously reported (McNamara *et al.*, 2007). In this regard, caffeine is a well-established sympathomimetic agent in humans, and excessive consumption elicits an increase in BP, raised circulating catecholamine levels and plasma renin activity (Benowitz *et al.*, 1995; Riksen *et al.*, 2009; Osswald and Schnermann, 2011), all indices of sympathetic nervous system activity. Moreover, Sondermeijer *et al.* (2002) reported that not only did caffeine consumption increase sympathetic tone in non-habitual caffeine users, it concurrently reduced parasympathetic nervous system activity, with an amplified shift in sympathovagal balance towards sympathetic predominance.

## Seizures

Derlet *et al.* (1992) first reported that co-administration of caffeine with amphetamine or cocaine led to a dramatic increase in seizures in rats compared with rats treated with either drug alone. In a similar way, caffeine provoked seizures in animals treated with MDA (McNamara *et al.*, 2006) or a combination of d-fenfluramine and d-amphetamine but not in animals treated with MDMA or equivalent doses of d-fenfluramine or d-amphetamine alone (Vanattou-Saïfoudine *et al.*, 2010a). McNamara *et al.* (2006) suggested that the lower toxicity threshold and increased potency of MDA compared with MDMA may at least partly account for the differences observed between the substituted amphetamines with respect to seizure occurrence. In addition, there are notable pharmacological differences between MDA and MDMA, including an increased propensity for MDA to induce dopamine release and to interact with 5-$HT_{2A}$ receptors (see Giorgi *et al.*, 2006), which may underlie the increased risk of seizures with this metabolite.

## Caffeine influences the stimulatory, discriminative and reinforcing effects of psychostimulant drugs of abuse

Caffeine-related dependence and interactions with psychostimulant drugs of abuse to promote dependence have been previously reviewed by Morelli and Simola (2011). Animal models of drug reinforcement and addiction have played an important role in gaining insight into these interactions.

Acutely, caffeine pre-exposure has been shown to dose-dependently augment the locomotor effects of cocaine (Schenk *et al.*, 1990) and d-amphetamine (Cauli *et al.*, 2003; Simola *et al.*, 2006). With regard to long-term caffeine exposure, rats chronically exposed to caffeine in their drinking water also show an increase in d-amphetamine and cocaine-induced ambulatory activity compared with rats drinking water without caffeine (Gasior *et al.*, 2000). More complex behaviour has also been assessed in schedule-controlled operant testing paradigms to determine the influence of caffeine on the actions of other psychostimulant drugs. Jaszyna *et al.* (1998) reported that chronic exposure to caffeine via drinking water sensitized rats to the behavioural effects of d-amphetamine and cocaine but not nicotine in a fixed-interval schedule of food reinforcement.

Acutely administered caffeine possesses dose-dependent discriminative stimulus effects in both humans (Mumford *et al.*, 1994) and rats (Mumford and Holtzman, 1991). Generalization to psychomotor stimulants including d-amphetamine, ephedrine and cocaine has been consistently reported in rats, and caffeine, in turn, has been shown to potentiate the discriminative stimulus effects of these drugs (Mumford and Holtzman, 1991). There are also reports that caffeine, administered to rats at 30 $mg \cdot kg^{-1}$ twice daily for 3.5 days, can induce a cross-tolerance to the discriminative effects of psychostimulant drugs including methylphenidate and the dopamine $D_1$ receptor agonist SKF 81297 and in some cases d-amphetamine, a phenomenon in which the dopamine $D_1$ receptor is proposed to play an important role. The same regimen of caffeine can also induce tolerance to the discriminative effects of caffeine in rats discriminating between caffeine and saline, suggesting that in some instances pharmacological tachyphylaxis rather than sensitization is achieved following repeated administration of caffeine (see Jain and Holtzman, 2005). Tolerance develops to the motor-activating effects of caffeine and formation of $A_1$-$A_{2A}$ receptor heteromers during chronic treatment with

caffeine have been proposed to play a role in mediating tolerance to the psychostimulant effects of caffeine (see Ferré *et al.*, 2008).

In the self-administration paradigm of drug reinforcement, caffeine alone does not reliably maintain self-administration behaviour in animals (Griffiths and Woodson, 1988). However, caffeine does potentiate the reinforcing effects of cocaine, whereby self-administration of cocaine is induced more rapidly, and cocaine self-administration already established is enhanced in association with increased cocaine-induced central dopamine release (Schenk *et al.*, 1994; Schenk and Partridge, 1999). Caffeine can also increase smoked cocaine self-administration in rhesus monkeys (Comer and Carroll, 1996). Moreover, pretreatment with caffeine dose-dependently reinstates extinguished cocaine self-administration like cocaine itself. This is interesting as it illustrates that caffeine can act as a 'priming agent', capable of inducing relapse to drug-seeking in an animal model in the absence of the actual drug being sought (Green and Schenk, 2002).

Conditioned place preference (CPP) is routinely used to demonstrate the rewarding effects of psychostimulant drugs in rats, including d-amphetamine and cocaine. Caffeine has also been tested in the CPP model and has been shown to have a dose-dependent effect: low doses of caffeine produce a significant place preference, while high doses have an opposite effect, and produce place aversion (Brockwell *et al.*, 1991). However, interactions between caffeine and other stimulants have not been widely investigated using this paradigm. While Bedingfield *et al.* (1998) demonstrated in rats that very low doses of cocaine and caffeine (0.32 $mg \cdot kg^{-1}$) had an additive effect on place preference, Poleszak and Malec (2002) showed that caffeine (10 and 20 $mg \cdot kg^{-1}$) attenuated both the acquisition and the expression of cocaine-induced CPP. However, these investigators subsequently showed that caffeine – at 10 $mg \cdot kg^{-1}$ only – potentiated d-amphetamine-induced expression of CPP (Poleszak and Malec, 2003), suggesting a dissociation between the neurochemical substrates underlying the reinforcing effects of d-amphetamine and cocaine.

Taken as a whole, these studies suggest that caffeine can augment the reinforcing, motor stimulant arousing and subjective effects of dopaminergic drugs of abuse, with implications for human drug users.

## Mechanisms and mediators associated with caffeine-related interactions with psychostimulants

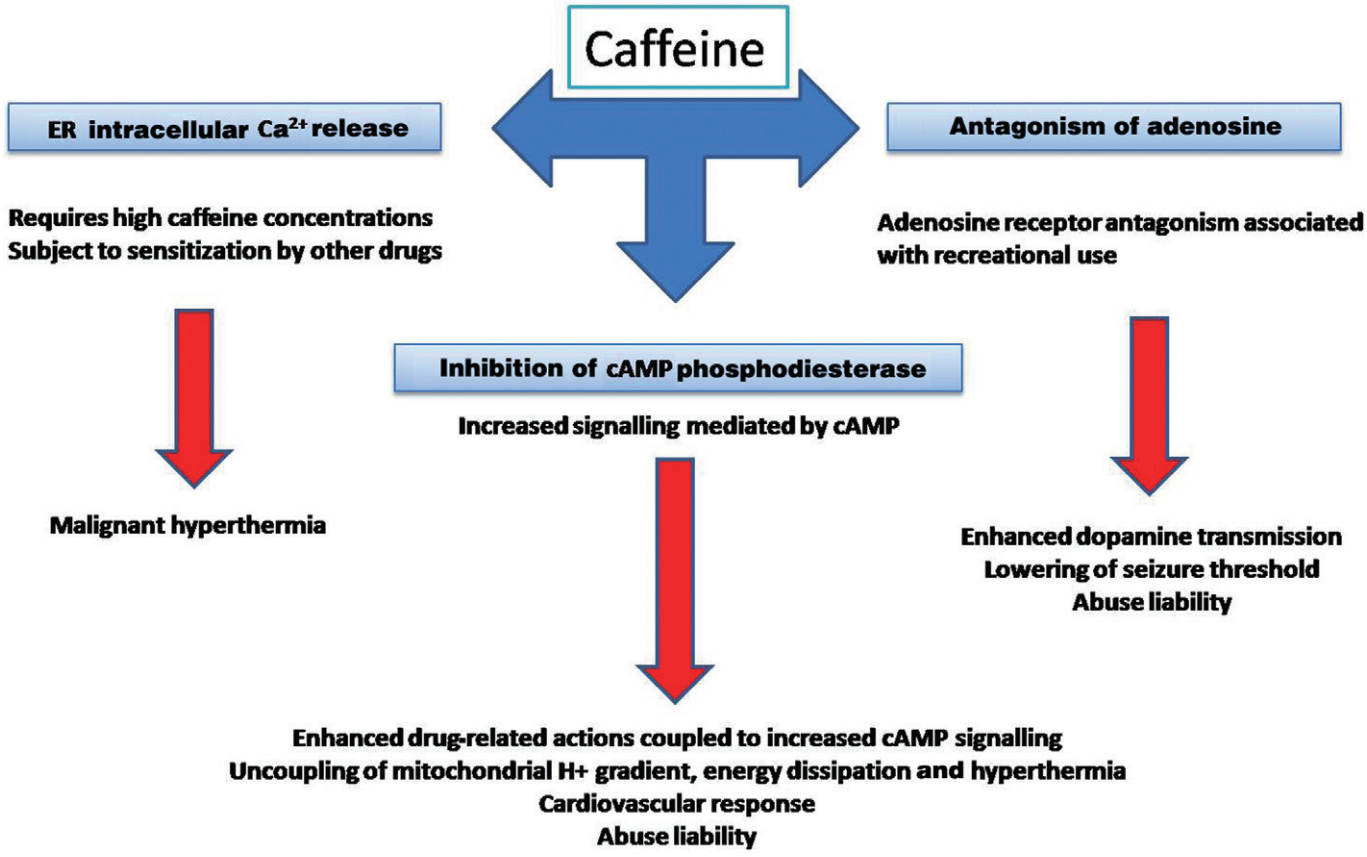
Reports on the effects of caffeine on core body temperature and other behavioural and physiological responses to a variety of psychostimulants in preclinical models have led to a number of mediators being proposed, which may account for the interactions observed. The main contributing biochemical actions of caffeine are highlighted in Figure 1.

### *Role of uncoupling ATP synthesis and thermogenesis*

The possibility exists that hyperthermia may result from heat generation in different tissue sites, as MDMA is a potent activator of the sympathetic nervous system and catecholamines are known to play a major role in heat production and distribution by altering vascular haemodynamics and influencing the output of peripheral thermogenic tissues, including brown adipose tissue (BAT), skeletal muscle and liver (Mills *et al.*, 2004).

BAT thermogenesis is under the control of cAMP and uncoupling protein (UCP)-1 (for review, see Richard and Picard, 2011) and this system is often used as a model to investigate the thermogenic effects of drugs. UCP-1 is a mitochondrial protein found in thermogenic tissues such as BAT and skeletal muscle, which has the ability to 'uncouple' ATP synthesis from dissipation of a generated proton gradient to produce heat. By contrast, its paralogues UCP-2 and UCP-3 are not thought to mediate whole body thermogenesis in mammals and, instead, play a role in a variety of physiological and pathological processes, including protection from oxidative stress, regulation of glucose sensing and fatty acid oxidation. A number of studies carried out in BAT have shown an interaction between caffeine and the stimulant drug ephedrine. Caffeine has been shown to activate BAT thermogenesis in mice and up-regulates the expression of UCP-1 and related subtypes -2 and -3 in BAT and UCP-2 and -3 in the skeletal muscle of obese mice (Kogure *et al.*, 2002). Ephedrine also induces BAT thermogenesis via sympathetically released noradrenaline. Combination of sub-threshold doses of ephedrine and caffeine or its metabolite paraxanthine results in a synergistic interaction and marked increases in BAT respiration rate, an index of BAT thermogenesis (Dulloo *et al.*, 1991; 1994). More importantly, it is thought that caffeine acts by augmenting ephedrine and adrenergic-associated elevations in intracellular cAMP via PDE inhibition and not adenosine receptor antagonism (Dulloo *et al.*, 1992; Dulloo, 1993). Interestingly, amphetamine also dose-dependently induces BAT thermogenesis in rats, an effect that is prevented by β-adrenoceptor antagonism (Tariq *et al.*, 1989) and, hence, the downstream activation of cAMP. Furthermore, it has been shown that MDMA activates UCP in skeletal muscle, probably due to enhanced cAMP levels resulting from thyroid and adrenergic stimulation, an effect that has been linked to hyperthermia (reviewed by Mills *et al.*, 2004). These studies therefore provide supporting evidence in favour of a cAMP-mediated interaction between caffeine and stimulant drugs that affects body temperature regulation.

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, which is induced by an uncontrolled increase of skeletal muscle oxidative metabolism (Rosenberg *et al.*, 2007). Such a mechanism may be relevant in light of animal and human studies, which have reported that MDMA intoxication and hyperthermia is associated with an elevation in myoplasmic calcium concentrations (Rusyniak *et al.*, 2004). Circulating peak concentrations of caffeine (10 $mg \cdot kg^{-1}$) following systemic administration fall between 30 and 40 μM in the rat. Indeed, circulating caffeine concentrations following caffeine ingestion in humans rarely exceed 100 μM. While caffeine can mobilize intracellular calcium, such a mecha-




## Figure 1

Concentration-dependent effects of caffeine in ascending order: (i) inhibition of adenosinergic activity; (ii) accumulation of intracellular cAMP; and (iii) release of calcium ($Ca^{2+}$) from intracellular stores [from endoplasmic reticulum (ER) ] which may contribute to caffeine-related toxicity.

nism is unlikely to be applicable either to human consumption or in the animal experiments described, as a minimum concentration of 250 μM is necessary to generate detectable effects on calcium shifts (see Endo, 2009). Moreover, caffeine does 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. In support of such a view, Klingler *et al.* (2005) reported a sensitization by MDMA of caffeine-induced contractures in skeletal muscle cells. This is of special clinical relevance as skeletal muscle is thought to be a peripheral target of MDMA and to play a role in the hyperthermic response to the drug. Nevertheless, as structurally related xanthines which influence the ryanodine receptor, including 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and pentoxifylline failed to influence MDMA-induced hyperthermia, it is likely that the principal mechanism by which caffeine influences MDMA-induced hyperthermia is via antagonism of adenosine receptors coupled to the inhibition of PDE discussed below (see Vanattou-Saïfoudine *et al.*, 2011).

### *Dual role for 5-HT and dopamine*

The UCP hypothesis suggests a peripheral mechanism of interaction that is probably confined to body temperature alterations. In order to generalize to other types of toxicity, it is necessary to look at the central and neural mechanisms of interaction. A common feature of all drugs that possess the potential for a harmful interaction with caffeine is the ability to affect dopaminergic neurotransmission. It is well established that caffeine has effects on central dopamine release, in addition to blocking negative interactions between adenosine $A_1$/dopamine $D_1$ and adenosine $A_2$/dopamine $D_2$ receptors as described in striatal and limbic brain regions (Cauli and Morelli, 2005; Ferré, 2010). Such effects strongly implicate dopamine in the interactions (temperature, cardiovascular and seizure threshold) elaborated upon earlier. In addition, the PDE inhibitory ability of caffeine may also drive the interactive effects with central dopaminergic transmission through enhancement of cAMP signalling.

Both dopamine $D_1$ and $D_2$ receptor subtypes are implicated in MDMA-induced changes in body temperature with $D_1$ receptor activation associated with hyperthermia, whereas dopamine $D_2$ receptor stimulation predominates in animals housed individually or at low ambient temperatures (see Green *et al.*, 2005). 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. In support of this, in individually housed animals, MDMA-induced hypothermia is enhanced by prior treatment with the dopamine $D_1$ receptor antagonist SCH 23390, which, in turn, blocks the ability of caffeine to promote a switch from hypo- to hyperthermia following MDMA administration. Furthermore, MDMA-induced hypothermia is blocked following pretreatment with the dopamine $D_2$ receptor antagonist sulpiride, which also attenuates the ability of caffeine to promote MDMA-induced hyperthermia (Vanattou-Saïfoudine *et al.*, 2010b). Such an attenuation with sulpiride suggests that post-synaptic dopamine $D_2$ receptor activation may also be necessary to enable a full manifestation of dopamine-mediated hyperthermia.

A role for 5-HT is also implicated where the co-administration of caffeine with a combination of d-fenfluramine and d-amphetamine provokes a hyperthermic response, similar to that described following the co-administration of caffeine with MDMA. Pretreatment with the preferential 5-$HT_2$ receptor antagonist ketanserin attenuates the hyperthermic response to MDMA and its exacerbation by caffeine. Co-administration of the 5-HT- and dopamine-selective agonists, dimethoxy-4-iodophenyl-aminopropane hydrochloride and apomorphine, respectively, with caffeine provokes hyperthermia but not when either agonist is administered with caffeine alone (Vanattou-Saïfoudine *et al.*, 2010a). MDMA has direct agonist actions at 5-HT receptors, which may account for its ability to provoke toxicity. Such actions include the ability of 5-HT receptors to influence dopamine release as 5-$HT_2$ receptors play an important role in the regulation of central dopaminergic function (for review, see Di Matteo *et al.*, 2008; Gudelsky and Yamamoto, 2008).

A role for dopamine $D_1$ and $D_2$ receptors in mediating changes in heart rate to caffeine and MDMA alone, and in combination, has also been proposed. It has been established that dopamine plays an important role in cardiovascular homeostasis mediated through α- and β-adrenergic receptors but also through dopamine $D_1$ and $D_2$ receptors, which are found on the heart and in the vascular system and play a role in the regulation of heart rate (Jose *et al.*, 2003; Zeng *et al.*, 2007). In the rat model established, pretreatment with the dopamine $D_1$ receptor antagonist SCH 23390 blocked the ability of caffeine to promote a tachycardic response to MDMA. By contrast, pretreatment with the selective dopamine $D_2$ receptor antagonist sulpiride resulted in a reduced tachycardic response but failed to block the ability of caffeine to promote tachycardia following co-administration with MDMA. Overall, the results suggest that the caffeine-provoked switch to tachycardia requires the activation of both dopamine receptor subtypes in order to induce the interactive response following the co-administration of caffeine and MDMA (see Vanattou-Saïfoudine *et al.*, 2010b). Other neurotransmitters such as noradenaline may also be involved, but further experiments are necessary to clarify such mechanisms. Moreover, additional investigations are warranted regarding changes in BP and the mechanisms associated with such changes following co-administration of these agents.

Caffeine, although largely lacking the reinforcing effects or abuse potential associated with drugs of abuse, may be capable of priming reward-relevant circuitry used by other reinforcing agents. Caffeine induced significant cross-tolerance to the amphetamine-like discriminative effects of the dopamine $D_1$ receptor agonist SKF 81297 in rats, suggesting that the dopamine $D_1$ receptor plays a role in these discriminative stimulus effects (Jain and Holtzman, 2005). Moreover, there is a direct evidence that the dopamine $D_1$ receptor mediates the reinstating effect of caffeine on extinguished cocaine self-administration behaviour (Green and Schenk, 2002).

Dopamine $D_1$ and $D_2$ receptors have opposing actions on the activity of adenylate cyclase (AC) and PKA, which converge on several biochemical markers. One of these is the dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), which is abundant in neurons receiving dopaminergic input. Activation of Protein Kinase A (PKA) and the consequent phosphorylation of DARPP-32 on the Thr34 residue occur in response to stimulation of dopamine $D_1$ receptors. In contrast, stimulation of dopamine $D_2$ receptors results in inhibition of PKA activation, the activation of protein phosphatase 2B and the consequent dephosphorylation of DARPP-32 (for details, see Girault and Greengard, 2004). In DARPP-32 knockout mice, the psychomotor stimulatory effects of caffeine are reduced. Furthermore, phosphorylation of DARPP-32 at Thr 35 in mouse striata is increased through inhibition of protein phosphatase-2A activity, supporting a role for DARPP-32 in mediating the psychomotor actions of caffeine (Lindskog *et al.*, 2002). Thus, the phosphorylation status of DARPP-32 provides an intra-neuronal marker of relative dopamine $D_1/D_2$ receptor activation and therefore may be useful to study the influence of caffeine on MDMA-induced intracellular changes associated with these receptors. Similarly, dopamine, through $D_1$ receptor activation, stimulates AC and therefore increases the activity of PKA leading to cAMP response element binding protein (CREB) phosphorylation (Neve *et al.*, 2004). In a study where alterations in hypothalamic intracellular markers were assessed, increased phosphorylation of DARPP-32 and CREB was observed only when caffeine was administered with MDMA and not following administration of either drug alone. Interestingly, pretreatment with SCH 23390 attenuated these changes in p-CREB and p-DARPP in the hypothalamus, suggesting that these intracellular changes are brought about by a convergence of mechanisms associated with the co-administration of both agents, with dopamine $D_1$ over $D_2$ receptor activation predominating (Vanattou-Saïfoudine *et al.*, 2012).

## *A role for adenosine receptor blockade and PDE inhibition*

Under normal physiological conditions, the mechanism of action of caffeine is primarily accounted for by antagonism of adenosine receptors (Fredholm *et al.*, 1999; Ferré, 2010). Studies with adenosine $A_1$ and $A_{2A}$ receptor knockout mice have provided evidence that, despite similar affinity of caffeine for these receptors, both the psychomotor stimulatory and the arousal effects of caffeine are mediated by the adenosine $A_{2A}$ receptor, with the $A_1$ receptor playing a minor role (for review, see Chen *et al.*, 2010). In rats, at high doses,

caffeine induces a modest hyperthermia, an increase in motor activity and increases in BP and heart rate. These effects of caffeine are mediated primarily through blockade of adenosine $A_1$ and $A_2$ receptors. Antagonistic adenosine $A_1$-$D_1$ 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 (Cauli and Morelli, 2005; Ferré, 2010). Thus, modulation of dopamine transmission through adenosine receptors is implicated in the effects of caffeine.

In studies conducted to date on the regulation of body temperature, co-treatment with adenosine antagonists including the non-selective $A_{1/2}$ receptor antagonist CGS 15943, the selective $A_1$ receptor antagonist DPCPX and the $A_{2A}$ receptor antagonist SCH 58261 failed to provoke a caffeine-like interaction with MDMA, indicating that blockade of adenosine receptors alone does not mediate this interaction between caffeine and MDMA (Vanattou-Saïfoudine *et al.*, 2010a). These findings prompted further investigation into the possibility that the PDE inhibitory effects of caffeine may play a role. It has been reported that effects of caffeine on PDE are of little relevance at the concentrations of caffeine administered *in vivo* (Fredholm *et al.*, 1999), although 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 *et al.* extensively investigated the effects of caffeine on thermogenesis induced by the stimulant 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 underlying this interaction, PDE inhibition, and not adenosine receptor antagonism, resulted in a potentiation of the effects of ephedrine by caffeine (Dulloo *et al.*, 1991; 1992; 1994). However, in contrast to these interactions with ephedrine, co-treatment with PDE inhibitors, including the non-selective PDE inhibitor pentoxifylline, the PDE-4 inhibitor rolipram and the PDE-5 inhibitor zaprinast fails to provoke a caffeine-like interaction with MDMA. Co-administration of a combination of MDMA with rolipram and CGS 15943 or SCH 58261, but not DPCPX, exacerbates MDMA-induced hyperthermia (Vanattou-Saïfoudine *et al.*, 2010a). Thus, inhibition of PDE coupled to adenosine $A_{2A}$ receptor blockade provokes a caffeine-like interaction with MDMA, suggesting that these targets can account for the exacerbation of MDMA-induced hyperthermia by caffeine.

It is possible that the observed cardiovascular effects resulting from caffeine and MDMA co-administration are also due to a combination of dopamine $D_1$ receptor stimulation, adenosine receptor antagonism and PDE inhibition, leading to alterations in ion movement, neuronal excitability and synaptic transmission via cAMP. However, although the dopaminergic receptor antagonists SCH 23390 and haloperidol have been shown to attenuate some of the cardiovascular effects of methamphetamine in squirrel monkeys (Schindler *et al.*, 1992), and more recently, MDMA, in rats (Vanattou-Saïfoudine *et al.*, 2010b), the observed effects of caffeine on MDMA-induced cardiovascular changes in the rat are thought to be largely due to the sympathomimetic properties of caffeine. Such a mechanism has also been reported to mediate the cardiovascular effects of cocaine (see Schindler *et al.*, 1995). As noradrenaline and adrenaline play an important role in cardiovascular functioning, it is likely that this neurotransmitter system is responsible for cardiac or pressor interactions that occur between caffeine and other stimulant drugs. For example, increased heart rate is primarily under the control of $\beta_1$-adrenoceptors, which are positively coupled to adenylate cyclase and, hence, cAMP production. Given the cAMP signalling pathway is utilized by this neurotransmitter system, PDE inhibition by caffeine consequently could play a role in drug-induced cardiovascular toxicity.

Adenosine acting through adenosine $A_1$ receptors acts as an endogenous anticonvulsant, which supports the notion that methylxanthines like caffeine have proconvulsant activity by antagonizing the function of endogenous adenosine. While the anticonvulsant role of adenosine $A_1$ receptors is well established, inhibition of adenosine $A_{2A}$ receptors may play a role in reducing susceptibility to seizures. Moreover, the effects of chronic dosing of caffeine can produce different effects where caffeine may reduce seizure liability yet in the absence of changes in adenosine $A_1$ or $A_{2A}$ receptors. A role for adenosine in mediating seizures associated with methylxanthines is reviewed in detail elsewhere (for review, see Boison, 2011). CNS stimulant intake can increase neuronal excitability leading to epileptogenesis, and in this regard, seizures have been reported as a cause of death in cases of both MDMA- and cocaine-related toxicity (Giorgi *et al.*, 2006; Devlin and Henry, 2008). Antagonism of the inhibitory influence of adenosine mediated through adenosine $A_1$ receptors promotes neuronal excitation. Consequently, co-administration of caffeine with other stimulant drugs may lower the seizure threshold, resulting in increased stimulant-induced neuronal discharge.

A role for adenosine, and in particular the adenosine $A_{2A}$ receptor, has been described in the initiation (Knapp *et al.*, 2001), maintenance (Soria *et al.*, 2006) and reinstatement of cocaine self-administration (Weerts and Griffiths, 2003) in a number of animal models. Adenosine $A_1$ and $A_2$ receptors have also been implicated in cocaine (Poleszak and Malec, 2002) and amphetamine (Poleszak and Malec, 2003)-induced CPP.

As adenosine $A_1$ receptors are directly involved in regulating pre-synaptic dopamine release, the effect of caffeine on MDMA-induced dopamine release in superfused tissue slices obtained from the striatum and hypothalamus pre-loaded with [$^3$H] dopamine has been described. When applied in combination, caffeine enhanced MDMA-induced dopamine release from striatal, but not hypothalamic, tissue slices. Additional experiments to further characterize the role of adenosine $A_1$ receptors in the effects of caffeine on MDMA-induced dopamine release have reported that DPCPX produces a caffeine-like action by provoking an increase in dopamine release following exposure to MDMA, while co-treatment of MDMA with the adenosine receptor agonist 2-chloro-*N*-cyclopentyladenosine attenuated MDMA-induced dopamine release (Vanattou-Saïfoudine *et al.*, 2011). Other investigators have also shown that caffeine increases dopamine and glutamate release in the striatum via the blockade of inhibitory pre-synaptic adenosine $A_1$ receptors (Borycz *et al.*, 2007). While it is of interest to assess the

influence of caffeine on MDMA-induced dopamine release, the importance of striatal dopamine release to the increased toxicity observed with caffeine administration is not clear. Moreover, adenosine $A_{2A}$ but not $A_1$ receptors are implicated in the increased hyperthermic response to the drug combination (see Vanattou-Saïfoudine *et al.*, 2010a).

As activation of the dopamine $D_1$ receptor increases the intracellular availability of cAMP, it is proposed that some facets of the interaction between caffeine and other psychostimulant drugs may be explained by an augmentation of $D_1$ receptor functioning made possible by the further accumulation of cAMP following caffeine-related adenosine receptor and/or PDE inhibition. A number of studies have used the PDE-IV inhibitor rolipram as a tool to specifically increase intracellular levels of cAMP and amplify the effect of agents already acting through a cAMP-dependent pathway. Several studies have demonstrated that rolipram profoundly affects dopaminergic-related behaviours and neuronal cell death (Iyo *et al.*, 1995; Sasaki *et al.*, 1995; Yamashita *et al.*, 1997; Mori *et al.*, 2000), providing evidence that PDE inhibition, and the resulting increase in intracellular levels of cAMP, can interact functionally with the dopaminergic system. West and Galloway (1996) showed that local intra-striatal infusions of rolipram increased basal dopamine levels to 143% above control levels. However, Iyo *et al.* (1996) did not observe an augmentation of methamphetamine-induced increases in intra-striatal dopamine levels by rolipram and concluded that any effects of rolipram on methamphetamine-induced behaviours were mediated postsynaptically. The possibility for postsynaptic interactions are further supported by a study showing that intracellular cAMP levels can affect dopamine $D_1$ receptor binding. Intra-striatal infusion of cAMP analogues dose-dependently increased *in vivo* binding of tritiated SCH 23390, which was prevented by pretreatment with an inhibitor of cAMP-dependent PKA (Abe *et al.*, 2002). Jiang and Sibley (1999) showed that PKA phosphorylation alters $D_1$ receptor sensitivity. Abe *et al.* (2002) concluded that the microenvironment of the $D_1$ receptors might be modulated by cAMP-dependent mechanisms, thereby altering the binding properties of the receptors. Thus, caffeine-associated PDE inhibition and the resulting accumulation of intracellular cAMP may alter the properties of the dopamine $D_1$ receptor to influence its function. PDE inhibition alone, however, is not sufficient to mimic the interaction between caffeine and MDMA where, for example, a combination of PDE inhibition and adenosine receptor antagonism (specifically, $A_{2A}$) is required to mimic caffeine's ability to exacerbate MDMA-induced hyperthermia. As discussed earlier, it appears that interactions between adenosine $A_{2A}$ receptors with dopaminergic receptor activation and subsequent signalling through cAMP, which is further influenced by PDE inhibition, are required elements for hyperthermia to manifest. Further studies are required to clarify whether such an interplay of mediators could account for interactions between other psychostimulants and consequent adverse effects.

Caffeine has pharmacological actions in addition to adenosine receptor blockade, cAMP PDE inhibition and intracellular calcium mobilization. Caffeine acts as an antagonist at the benzodiazepine positive modulatory site of $GABA_A$ receptors and has been reported to inhibit TREK-1 K+ channels in a concentration- and cAMP-dependent manner, which plays a role in controlling neuronal excitability (reviewed by Boison, 2011). In both cases, however, submillimolar concentrations are required, which are not normally attainable by normal human caffeine consumption and are therefore not likely to account for the physiological effects of caffeine.

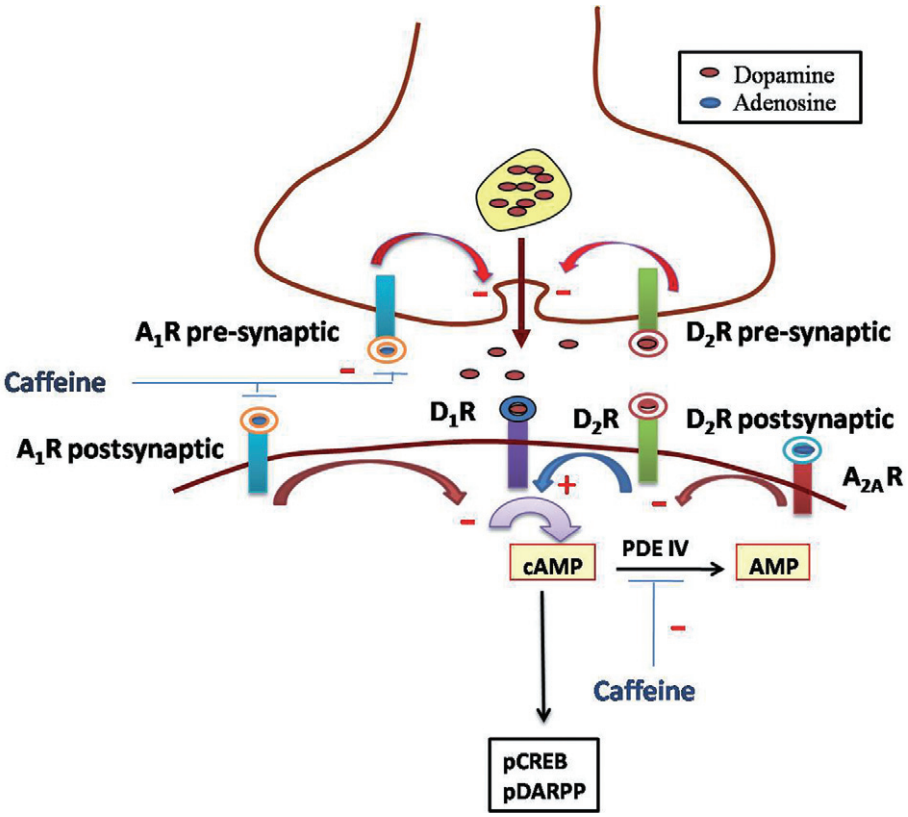
A proposed synaptic model depicting mediators underlying the interaction between caffeine and MDMA is illustrated in Figure 2.

### *Contribution of genetic variation in the population – CYP 450, adenosine receptors and dopamine receptors*

The influence of genetics on caffeine consumption and responses to caffeine has been recently reviewed (Yang *et al.*, 2010). Briefly, data generated from twin studies suggest that genetics play a role in levels of caffeine consumption and caffeine response including susceptibility to withdrawal effects. From the genetic association studies, variability in the CYP P450 complex, adenosine receptors and dopamine receptors can influence risk of certain diseases associated with caffeine consumption and some caffeine-related responses. In particular, human studies have reported that different adenosine $A_{2A}$ receptor and/or dopamine $D_2$ receptor polymorphisms are associated with caffeine-induced anxiety (Childs *et al.*, 2008). Further research is necessary to understand the influence of genetics on long-term consumption of caffeine and the functional significance of genotypes on interactions between caffeine and related psychostimulants.

## Conclusion

While the need for further research is evident, it is likely that concomitant consumption of caffeine with other stimulant drugs such as cocaine, d-amphetamine or MDMA can profoundly alter the drug response and drug-taking experience. In this regard, in cases of severe acute toxicity in particular, routine measurement of plasma caffeine concentrations in human drug users following hospital admission would help to more fully elucidate the potential of caffeine to initiate a severe reaction to other psychostimulant drugs of abuse. Experimental laboratory studies are needed to determine whether the ability of caffeine to influence the toxicity of MDMA generalizes to other amphetamine derivatives such as methamphetamine. As many of the drugs, examined to date, increase synaptic concentrations of dopamine, it is possible that the ability of caffeine to block adenosine receptors coupled to its PDE inhibitory properties interact with enhanced intracellular levels of cAMP following dopamine receptor activation to account for drug-related toxicity. An understanding of these underlying mechanisms will help to guide appropriate pharmacological treatment strategies for the management of severe toxicity associated with caffeine-related drug interactions. The mechanisms underlying the ability of caffeine to augment the abuse liability of psychostimulant drugs are also worthy of further consideration.




## Figure 2

Synaptic model of caffeine-MDMA interactions. MDMA stimulates 5-HT release and also provokes dopamine release, subsequent to 5-$HT_2$ receptor activation on the dopaminergic nerve terminal. Increased dopamine release subsequently leads to the activation of postsynaptic dopamine $D_1$ receptors, leading to $D_1$-mediated changes including hyperthermia. Caffeine may interact with this process by (i) facilitating dopamine release via a pre-synaptic adenosine $A_1$-dependent mechanism; (ii) inhibiting PDE and thereby inducing an accumulation of intracellular cAMP following dopamine $D_1$ receptor activation. With respect to hyperthermic responses, results to date would seem to favour the latter possibility as the influence of caffeine on MDMA-induced dopamine release was restricted to the striatum. Via inhibition of PDE, caffeine prevents cAMP metabolism and augments the activity of the PKA pathway, thereby increasing the phosphorylation of CREB and DARPP-32 in the hypothalamus. Additional mechanisms may also be possible, including (iii) inhibition of adenosine $A_{2A}$ receptors and (iv) prevention of intracellular cAMP breakdown following adenosine receptor blockade. It appears that both PDE inhibition and adenosine receptor antagonism in combination are required to mimic the hyperthermic interaction between caffeine and MDMA, suggestive of a synergistic interaction between these two pharmacological actions of caffeine. Activation of pre-synaptic dopamine $D_2$ receptors inhibits dopamine release, leading to opposing physiological responses to MDMA under specific conditions (i.e. hypothermia in singly housed rats or in low ambient temperatures). In this regard, it is proposed that co-administration with caffeine leads to a switch from dopamine $D_2$ receptor activation to promotion of a dopamine $D_1$ receptor-mediated response, consistent with an overall augmentation of dopaminergic transmission (v). With regard to postsynaptic dopamine $D_2$ receptors, co-administration with caffeine inhibits adenosine $A_{2A}$ receptors, which are physiologically negatively coupled with postsynaptic dopamine $D_2$ receptors, leading to an enhancement of dopamine $D_2$-related responses (vi). Postsynaptic dopamine $D_1$ and $D_2$ receptors operate synergistically in a number of synaptic regions (vii). The cascade of reactions signals the convergent inter- and intracellular points at which the pharmacodynamic actions of caffeine and MDMA may interact and which may account for the profound behavioural and physiological changes, which are characteristic of this drug interaction.

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## Conflicts of interest

The authors have no conflicts of interest to declare.

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