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The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A_{2A} receptors

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1 The locomotor stimulatory effects induced by caffeine (1,3,7-trimethylxanthine) in rodents have been attributed to antagonism of adenosine A_1 and A_{2A} receptors. Little is known about its locomotor depressant effects seen when acutely administered at high doses. The roles of adenosine A_1 and A_{2A} receptors in these activities were investigated using a Digiscan actimeter in experiments carried out in mice. Besides caffeine, the A_{2A} antagonist SCH 58261 (5-amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine), the A_1 antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine), the A_1 agonist CPA (N^6-cyclopentyladenosine) and A_{2A} receptor knockout mice were used.

2 Caffeine had a biphasic effect on locomotion of wild-type mice not habituated to the open field, stimulating locomotion at 6.25–25 mg kg^{-1} i.p. doses, while depressing it at 100 mg kg^{-1}. In sharp contrast, caffeine dose-dependently decreased locomotion in A_{2A} receptor knockout mice over the whole range of tested doses.

3 The depressant effects induced by high doses of caffeine were lost in control CD1 mice habituated to the open field.

4 The A_1 agonist CPA depressed locomotion at 0.3–1 mg kg^{-1} i.p. doses.

5 The A_1 antagonist DPCPX decreased locomotion of A_{2A} receptor knockouts and CD1 mice at 5 mg kg^{-1} i.p. and 25 mg kg^{-1} i.p. respectively.

6 DPCPX (0.2–1 mg kg^{-1} i.p.) left unaltered or even reduced the stimulant effect of SCH 58261 (1–3 mg kg^{-1} i.p.) on CD1 mice.

7 These results suggest therefore that the stimulant effect of low doses of caffeine is mediated by A_{2A} receptor blockade while the depressant effect seen at higher doses under some conditions is explained by A_1 receptor blockade.

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Abbreviations: SCH 58261, 5-amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; KF 17837, (E)-1,3-dipropyl-7-methyl-8-(3,4-dimethoxystyryl)xanthine; ZM 241385, 4-(2-[7-amino-2-furyl1,2,4triazolo2,3-a1,3,5 triazin-5-ylaminoethyl)phenol; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; CPA, N^6-cyclopentyladenosine; CHA, N^6-cyclohexyladenosine; R-PIA, R (−)-N^6-phenylisopropyladenosine; CCPA, 2-chloro-N^6-cyclopentyladenosine; DMPX, 3,7-dimethyl-1-propargylxanthine; DMSO, dimethylsulphoxide

Introduction

It is well established that, in the central nervous system, adenosine functions as a neuromodulator acting through discrete cell-surface receptors. Adenosine receptors were recognized more than 20 years ago, in part on the basis of the ability of caffeine (1,3,7-trimethylxanthine) to act as an antagonist at these receptors. Later, adenosine receptors were classified into two major subtypes called A_1 and A_2 (van Calker *et al*., 1979). Caffeine is likely to exert its primary action through the adenosine receptors, since they are the only known sites that bind caffeine at low concentrations (Fredholm, 1995). About 10 years ago, the first two adenosine receptors, A_1 and A_{2A}, were identified among putative G-protein coupled receptors, cloned from dog thyroid (Libert *et al*., 1989; 1991; Maenhaut *et al*., 1990). Later on, two other receptor types, A_{2B} and A_3, were cloned (Stiles, 1997). It is widely accepted that A_1, A_2 and even A_3 receptors may contribute to the ability of adenosine to modulate spontaneous locomotor activity (Jarvis, 1997). The possibility also exists that the activation of different adenosine receptor subtypes can synergistically contribute to locomotor suppression (Nikodijevic *et al*., 1991). The involvement of adenosine in regulating complex central functions, such as anxiety states (Jain *et al*., 1995; El Yacoubi *et al*., submitted) has also been widely investigated in the past.

Caffeine has important effects on alertness, and there is no doubt that caffeine is widely consumed by subjects who need to stay awake (Fredholm *et al*., 1999). Stimulant effects have been quantified in locomotor activity studies in rodents (Snyder *et al*., 1981; Svenningsson *et al*., 1997) or sleep studies in rats (Yanik *et al*., 1987; Schwierin *et al*., 1996). Numerous findings have prompted speculation that the behavioural effects of caffeine might be associated with its ability to block adenosine receptors (Fredholm, 1980; Snyder *et al*., 1981). Support for a central mechanism in the locomotor-stimulating effects of caffeine was derived from the observation that the activity of methylxanthine analogs correlated well with their ability to inhibit the binding of the selective A_1 receptor agonist [^{3}H]-

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CHA (N^6-cyclohexyladenosine) in brain (Snyder *et al*., 1981; Katims *et al*., 1983). Later on, the adenosine receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) that is 3 fold selective for binding to A_{2A} versus A_1 receptors (Jacobson & Van Rhee, 1997) was shown to be slightly more potent than caffeine in increasing locomotor activity of mice (Seale *et al*., 1988). In fact, caffeine causes biphasic effects on locomotion; low doses increasing and high doses decreasing locomotor activity (Boissier & Simon, 1965; Waldeck, 1975; Logan *et al*., 1986; Nikodijevic *et al*., 1993).

We have previously shown that the locomotor stimulant effects of one dose of caffeine (25 mg kg^{-1} i.p.) observed in wild-type mice were turned into locomotor depressant effects in A_{2A} receptor knockout mice; this showed the prominent role of acute blockade of the A_{2A} receptor in the locomotor stimulant action (Ledent *et al*., 1997). To strengthen this result, the purpose of experiment one was to compare the motor patterns elicited by different doses of caffeine or its vehicle in wild-type and A_{2A} receptor knockout mice.

Both novelty stress and caffeine share many features, namely an increase in locomotor activity (stress: Antelman *et al*., 1980; low doses of caffeine: Snyder *et al*., 1981) an increase in dopaminergic activity (stress: Abercrombie *et al*., 1989; caffeine: Ferré *et al*., 1997), and an increase in plasma corticosteroids (stress: Misslin *et al*., 1982; high doses of caffeine: Henry & Stephens, 1980; Spindel *et al*., 1983). In view of these facts, it became of interest to see whether locomotor responses to caffeine are affected by the stress of novelty. For that purpose, a second experiment was designed to investigate the locomotor response to caffeine in habituated and non-habituated wild-type mice.

The caffeine-induced locomotor depressant response in the A_{2A} receptor knockout mouse was suggested to be mediated by the blockade of the A_1 receptor (Ledent *et al*., 1997). A comparison of the locomotor responses produced by caffeine with those elicited by selective A_1 receptor ligands may help to reveal the role of this receptor in the caffeine-induced locomotor effects. N^6-cyclopentyladenosine (CPA) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), potent adenosine A_1 receptor agonist and antagonist respectively, display about 700 fold selectivity for A_1 versus the A_{2A} receptor (Jacobson & Van Rhee, 1997). Their effects were investigated here in the experimental settings used for caffeine in non-habituated wild-type mice since they have previously been used over a broad range of doses in motor activity studies (Marston *et al*., 1998). The activation of nigro-striatal pathways may be involved in the vertical component of locomotor activity i.e. rearing (Al-Khatib *et al*., 1995 and references therein). Since the effects of DPCPX upon the rearing behaviour displayed interesting features in one study performed in rats (Svenningsson *et al*., 1997), the purpose of experiment four was to record the effects of the selective A1 receptor antagonist upon this behaviour in wild-type and A_{2A} receptor knockout mice.

Finally, we also examined the effects induced by combinations of a selective A_{2A} antagonist and a selective A_1 antagonist in wild-type mice. Besides DPCPX, the selective A_{2A} antagonist used was 5-amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261), a highly potent and almost 500 fold selective for A_{2A} versus A_1 receptor (Ongini, 1997). This last experiment tested the hypothesis that A_1 receptor blockade might exert facilitatory effects upon the stimulant locomotor effects elicited by A_{2A} receptor antagonism as proposed in another study (Jacobson *et al*., 1993).

Thus, through extending the test conditions and using A_{2A} receptor knockout mice, the present experiments were designed to provide a wider picture of the effects of caffeine on locomotor activity.

Methods

Animals

Male Swiss albino CD1 mice (Charles River, Saint Aubin lès Elbeuf, France) or A_{2A} receptor knockout mice and their wild-type controls bred on a CD1 background (Ledent *et al*., 1997), weighing 20–30 g were used at least after 1 week of habituation in our own facilities. Mice were housed in groups of 15–20 in Makrolon cages (38 × 24 × 18 cm) with free access to water and food (U.A.R., France) and kept in a ventilated room at a temperature of 21°C ± 1°C, under a 12 h light/12 h dark cycle (light on between 7 a.m. and 7 p.m.). Experiments were carried out between 9 a.m. and 7 p.m. The animals were isolated in small individual cages (27 × 13 × 13 cm) for 30 min prior testing.

The procedures described comply with ethical principles and guidelines for care and use of laboratory animals adopted by the European Community, law 86/609/CCE.

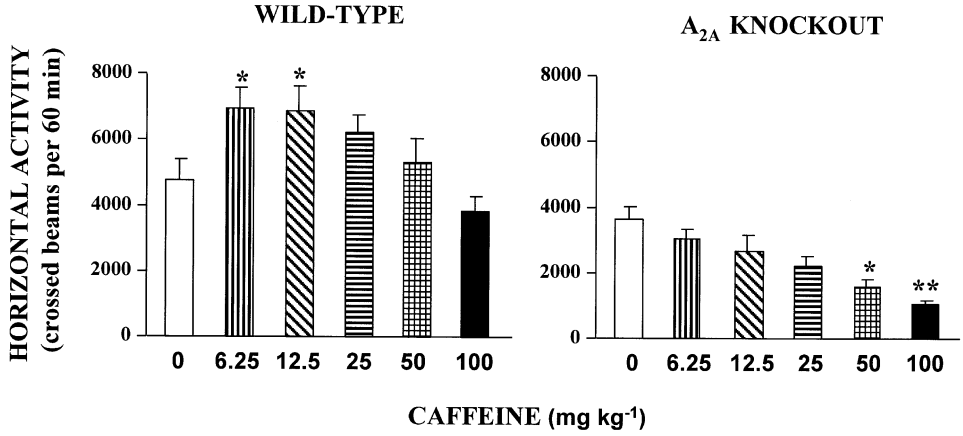

Figure 1 Effects of increasing doses of caffeine on locomotor activity in non-habituated wild-type and A_{2A} receptor knockout mice. Mice were injected with vehicle or increasing doses of caffeine (6.25–12.5–25–50–100 mg kg^{-1} i.p.) and were introduced into the actimeters. The horizontal component of locomotor activity was measured for 60 min. Means ± s.e.mean of data from ten controls and ten mice in treated groups. $^*P<0.05$, $^{**}P<0.01$ as compared to respective vehicle groups (Newman-Keuls *post hoc* test following a one-way ANOVA).

Locomotor activity

Locomotor activity was measured with a Digiscan Animal Activity Monitor system (Omnitech Electronics Inc., Columbus, OH, U.S.A.) which monitored the horizontal (locomotion) and vertical (rearing) movements of the animals. The Digiscan analyser was interfaced with a IBM-PC compatible computer using Digipro software. The individual compartments (L = 20; W = 20; H = 30 cm) were put in a dimly lit and quiet room. Horizontal i.e. locomotion and vertical movements i.e. rearing were expressed as a number of beams crossed over three (experiments with CPA or DPCPX alone) or four (experiments with caffeine or DPCPX + SCH 58261) 15 min periods of testing.

Testing conditions

A_{2A} receptor knockout mice and their wild-type controls (8–10 animals per group) bred on a CD1 background were used in experiments one and four (see Figure 1 and Table 1). Other experiments were performed using CD1 mice (11–17 animals per group).

Except in experiment two, mice were introduced into the actimeters without habituation to the test open field i.e. were non-habituated mice. Naive non-habituated mice injected with vehicle (10 ml kg^{-1} i.p.) and placed immediately into the unfamiliar test environment exhibited exploratory locomotion (horizontal activity) throughout the experiment, interspersed by increasing periods of stillness (see for example Results Figure 2, left panel).

In experiment two (see Results Figure 2, right panel), groups of naive CD1 mice were first thoroughly habituated to the test environment over a 30 min period. Then, they were removed from the open field, injected with increasing doses of caffeine (6.25–100 mg kg^{-1} i.p.) or its vehicle, and replaced in the actimeters for an additional 60 min. This habituation procedure was applied so that the activity of the subjects in the motility cages could be recorded during the subsequent experimental period without the interference of spontaneous exploratory behaviour.

In experiment five, CD1 mice were pretreated with vehicle or DPCPX (0.2–1 mg kg^{-1} i.p.) 15 min prior to the acute administration of vehicle or SCH 58261 (1–3 mg kg^{-1} i.p.). Immediately after the last treatment, they were introduced into the actimeters for a 60 min test.

Drugs

Caffeine (1,3,7-trimethylxanthine), DPCPX (8-cyclopentyl-1,3-dipropylxanthine) and CPA (N^6-cyclopentyladenosine) were purchased from RBI (Natick, MA, U.S.A). SCH 58261 (5-amino-7-(β-phenylethyl)-2-(8-furyl)pyrazolo[4,3-e]-1, 2, 4-triazolo[1,5-c]pyrimidine) was a generous gift from Dr E. Ongini (Schering-Plough Research Institute, Milan, Italy). Caffeine (6.25–100 mg kg^{-1}) was dissolved in an aqueous solution of sodium benzoate (10 mg ml^{-1}). DPCPX (0.2–1–5–25 mg kg^{-1}), CPA (0.03–0.1–0.3–1 mg kg^{-1}) and SCH 58261 (1–3 mg kg^{-1}) were dissolved in dimethyl sulphoxide (Sigma) and then diluted in Cremophor EL (Sigma) and NaCl 0.9% (final concentration: 15% DMSO and 15% Cremophor EL). The solutions of drugs were prepared fresh daily and injected i.p. in a volume of 10 ml kg^{-1}.

Table 1 Effects of DPCPX on locomotor activity in non-habituated wild-type and A_{2A} receptor knockout mice

Mice	*Wild-type*		*A_{2A} Receptor knockout*	
drug	*Vehicle*	*DPCPX*	*Vehicle*	*DPCPX*
HLA	4921 ± 212	4570 ± 462	3887 ± 253$^{\#\#}$	2727 ± 304*
VLA	797 ± 75	795 ± 215	538 ± 66$^{\#}$	211 ± 52**

Mice were injected immediately before testing with vehicle or DPCPX (5 mg kg^{-1} i.p.). Horizontal (HLA) and vertical (VLA) components of locomotor activity (crossed beams) were recorded for 45 min. Means ± s.e.m. of data from 8 mice per group. *$P<0.05$; **$P<0.01$ as compared to respective vehicle treated group; #$P<0.05$; ##$P<0.01$ as compared to vehicle-injected wild-type mice (Student's *t*-test).

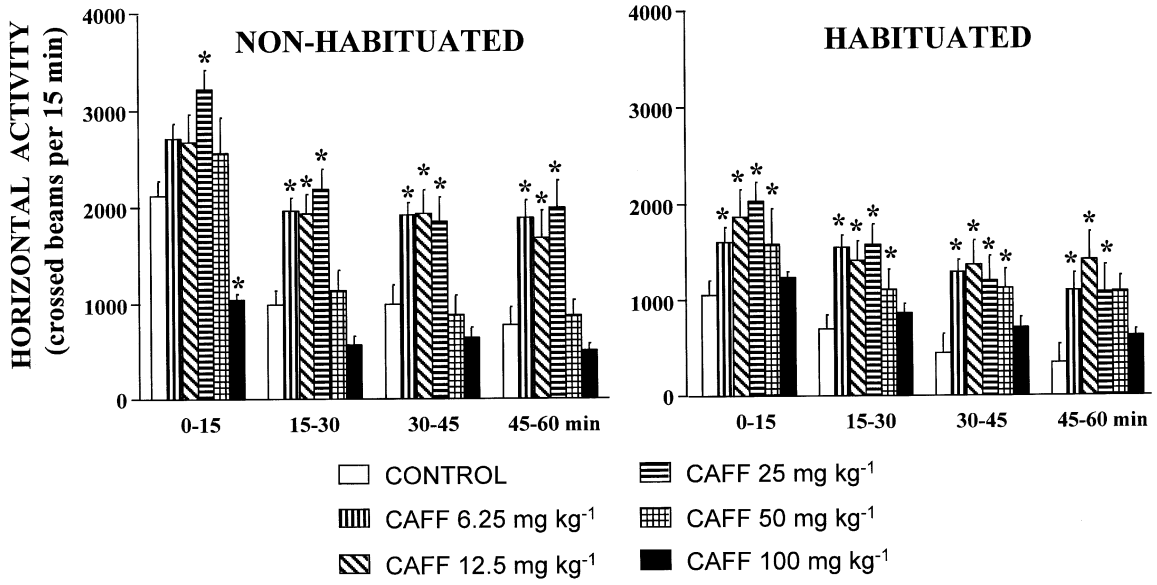

Figure 2 Effects of increasing doses of caffeine on locomotor activity in non-habituated and habituated CD1 mice. Mice were injected with vehicle or increasing doses of caffeine (doses and symbols are the same as in Figure 1) and were introduced into the actimeters. The horizontal component of locomotor activity were measured for 60 min without (left panel) or with (right panel) habituation to the test open field. Means ± s.e.mean of data from 14 controls and 12–13 mice in treated groups. *$P<0.05$ (by Newman-Keuls *post hoc* test following a one-way ANOVA).

Statistics

Results are expressed as means ± s.e.mean. Statistically, these data were tested first within a group of experiments (i.e. habituated or non-habituated mice) by means of three-way ANOVAs for repeated measures, using time of testing (three or four levels) and doses as between factors, and subjects as within factors. The repeated measures of locomotor activity during a testing session were considered the within-subject-dependent repeated measure. When the effects were time-dependent (significant time × dose interaction), separate ANOVAs were performed at each period of testing. *Post hoc* analysis of significant effects were performed using either Newman-Keuls tests for multiple comparisons, to compare treated groups versus the respective control group, or Student's *t*-test to compare two groups. Concerning experiment two, in a second level of data analysis, we asked whether the locomotor effects of caffeine obtained after habituation to the Digiscan apparatus differed from those observed in non-habituated mice. Therefore, we compared the two experiments using an additional ANOVA for repeated measures, using 'habituation' (two levels) and drugs as between factors, and animals as within factor. Significance levels were set at $P<0.05$.

Results

Effects of caffeine on locomotor activity in non-habituated wild-type and A_{2A} knockout mice

The locomotion of wild-type mice, injected with either vehicle or increasing doses of caffeine (6.25 – 100 mg kg^{-1} i.p.), has been compared with the locomotion of the A_{2A} receptor knockout mice, following similar drug injections (Figure 1). Of importance in this comparison was the significant interaction between caffeine and the mutation conditions revealed by a two-way ANOVA ($F(5,119)=2.78$, $P<0.05$). Separate one-way ANOVAs followed by Newman-Keuls *post hoc* tests indicated that caffeine effects differed significantly in wild-type and A_{2A} receptor knockout mice at every tested dose. In wild-type control mice, the acute administration of caffeine induced biphasic effects: a significant increase in locomotion was observed at low doses (6.25 – 12.5 mg kg^{-1}) and this stimulant effect gradually subsided at higher doses. By contrast, it induced a dose-dependent decrease in locomotion over the whole range of tested doses in A_{2A} receptor knockout mice (Figure 1).

Effects of caffeine on locomotor activity in non-habituated and habituated CD1 mice

The results of the experiment are summarized in Figure 2. The acute caffeine administration (6.25 – 12.5 – 25 – 50 – 100 mg kg^{-1} i.p.) induced motor effects that varied with time as shown by dose × time interactions in three-way repeated measures ANOVAs for non-habituated mice (locomotion: [$F(15,295)=3.01$, $P<0.001$)]; rearing: $F(15,295)=3.01$, $P<0.001$]) and habituated mice (locomotion: $F(15,315)=2.29$, $P<0.01$); rearing: $F(15,315)=2.03$, $P<0.05$)]. First, in non-habituated mice, locomotion was initially decreased for 15 min at an intermediate dose of caffeine (25 mg kg^{-1} i.p.) but decreased at the highest tested dose (100 mg kg^{-1} i.p.) in comparison to control mice. The three lowest doses (6.25 – 12.5 – 25 mg kg^{-1} i.p.) induced a stimulation of activity above control levels for the remainder of the experiment (Figure 2, left panel). The effects of caffeine on rearing in non-habituated mice were roughly similar to those on locomotion, i.e. biphasic and dose-dependent. However, the reduction of rearing observed at 100 mg kg^{-1} did not reach a statistically significant level (data not shown). Second, in habituated mice, a decrease of locomotion with time was observed for the vehicle-injected mice that displayed some motor activity during the first 15 min period, due to the handling and injection procedure. The absence of a clear dose-dependent effect upon locomotion for the doses ranging from 6.25 up to 50 mg kg^{-1} suggested that the maximum stimulant effect of caffeine already occurred at the lowest tested dose (6.25 mg kg^{-1}) in this experimental condition. Furthermore, in habituated animals, caffeine (100 mg kg^{-1}) induced no changes in locomotion during the whole duration of the experiment as compared to vehicle. Very little rearing was recorded in vehicle-injected mice during the 1 h test period. Caffeine (from 6.25 up to 25 mg kg^{-1}) enhanced vertical activity at least during the first half-hour after injection (data not shown).

Comparison between habituated and non-habituated CD1 mice

Figure 2 shows that horizontal photocell counts elicited in the non-habituated vehicle group were largest during the first 15 min after injection and progressively decreased down to the level observed for habituated mice by the end of the measurement period. Data obtained from non-habituated and habituated mice were compared using an additional ANOVA for repeated measures over time using habituation as a between factor. A significant interaction was obtained between habituation and caffeine factors on locomotion at each sampling period [0 – 15 min: $F(5,152)=3.97$, $P<0.01$; 15 – 30 min: $F(5,152)=2.29$, $P<0.05$; 30 – 45 min: $F(5,152)=2.71$, $P<0.05$; 45 – 60 min: $F(5,152)=3.57$, $P<0.01$] indicating that habituation to the test environment influenced the behaviour of mice injected with the entire dose range of caffeine (6.25 – 100 mg kg^{-1}) during the early period post-injection and, most interestingly, the behaviour of mice injected with the two highest doses throughout the whole test session.

Role of adenosine A1 receptors in mediating locomotor activity changes

1 receptor agonist CPA (0.03 – 0.1 – 0.3 – 1 mg kg^{-1} i.p.) induced time-dependent effects on locomotion (time × dose interaction: $F(8,221)=2.20$, $P<0.05$) in CD1 mice. Devoid of effects at low doses, CPA (0.3 – 1 mg kg^{-1} i.p.) significantly decreased photocell counts at 0.3 and 1 mg kg^{-1} (Figure 3, upper panel). A significant decrease in rearing behaviour was only evidenced with 1 mg kg^{-1} of CPA (data not shown).

The selective A_1 adenosine receptor antagonist DPCPX (0.2 – 1 – 5 – 25 mg kg^{-1} i.p.) induced a time-dependent, short lasting i.e. 30 min (dose × time interaction: $F(8,191)=4.64$, $P<0.001$), depressant effect on locomotion when administered at 25 mg kg^{-1} (Figure 3, lower panel). In this experiment, a decrease of vertical activity was elicited by DPCPX (25 mg kg^{-1}) that did not reach a significant level (data not shown).

To further assess the involvement of the A_1 receptor in the locomotor depressant activity observed in caffeine-treated

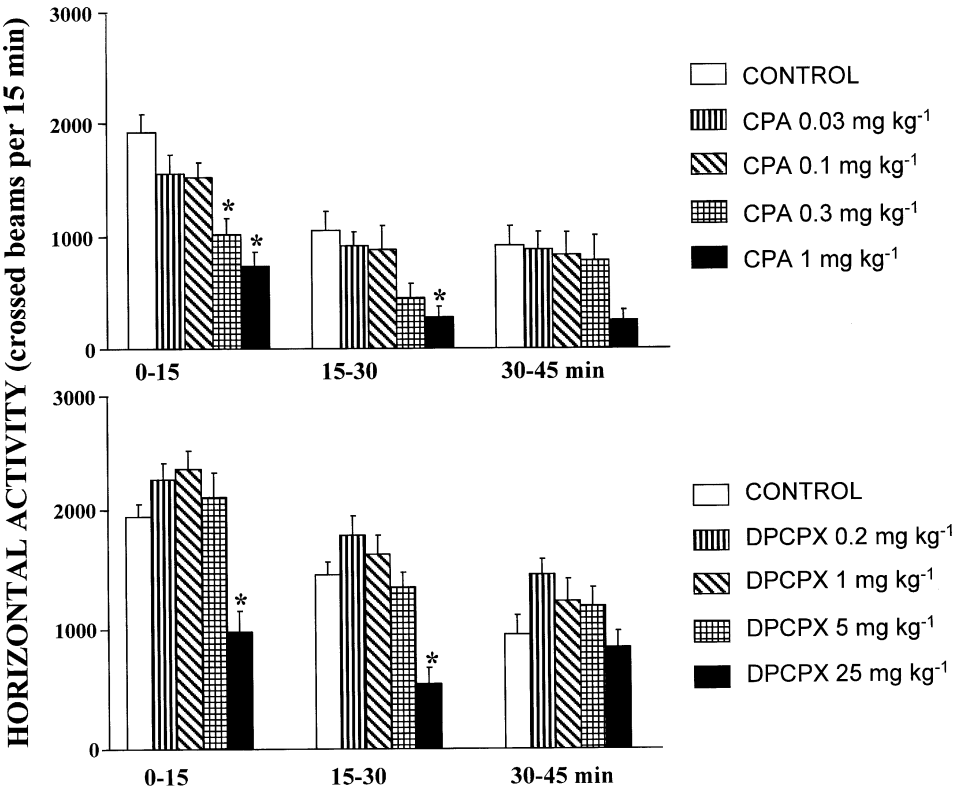

Figure 3 Effects of increasing doses of CPA or DPCPX on locomotor activity in non-habituated CD1 mice. Mice were injected with vehicle or increasing doses of CPA (0.03–0.1–0.3–1 mg kg^{-1} i.p.) or DPCPX (0.2–1–5–25 mg kg^{-1} i.p.) and were introduced into the actimeters. The horizontal component of locomotor activity was measured for 45 min. Means ± s.e.mean of data from 14 controls and 13–15 mice in treated groups. *$P<0.05$ (by Newman-Kreuls *post hoc* test following a one-way ANOVA).

animals, wild-type controls and A_{2A} receptor knockout mice were acutely treated with 5 mg kg^{-1} DPCPX. At this moderate dose, the selective A_1 receptor antagonist significantly decreased the horizontal locomotion and the rearing activity in A_{2A} receptor knockout mice but not in wild-type animals (Table 1).

Role of adenosine A_{2A} receptors in mediating locomotor activity changes

The selective A_{2A} antagonist SCH 58261 induced a dose-dependent (1–3 mg kg^{-1} i.p.) increase in locomotion immediately after its injection to non-habituated CD1 mice (separate ANOVA followed by multiple comparisons using Newman-Kreuls *post hoc* test). DPCPX (0.2–1 mg kg^{-1} i.p.), when administered alone 15 min prior testing, caused a slight increase in horizontal locomotor activity in this experiment. A two-way ANOVA analysis revealed a significant interaction between DPCPX pretreatment and SCH 58261 treatment upon locomotion [$F(4,134)=3.84$, $P<0.01$]. At 0.2 mg kg^{-1}, DPCPX attenuated the stimulant locomotor effects induced by SCH 58261, whereas a more complex interaction occurred when DPCPX was administered at 1 mg kg^{-1} (Figure 4).

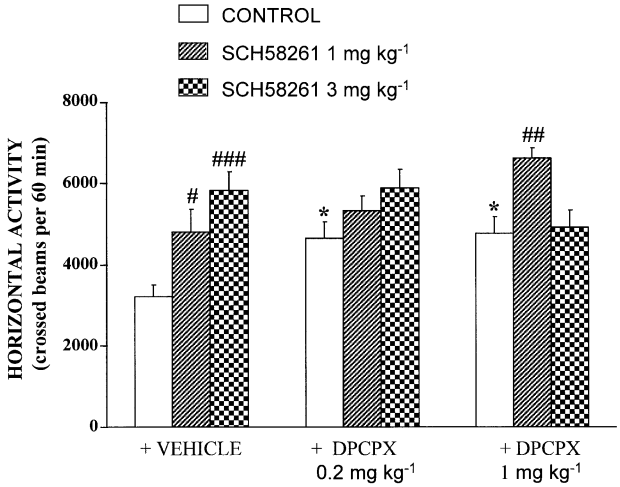

Figure 4 Effect of DPCPX on stimulatory locomotor activity induced by the A_{2A} receptor antagonist SCH 58261 in non-habituated CD1 mice. Mice were injected with vehicle or DPCPX (0.2–1 mg kg^{-1} i.p.). Fifteen minutes later, they were injected with vehicle or SCH 58261 (1–3 mg kg^{-1} i.p.) and were introduced into the actimeters. The horizontal component of locomotor activity was measured for 60 min. Data are means ± s.e.mean for groups of 17 controls and 11–17 mice in treated groups. *$P<0.05$ as compared with respective DPCPX-untreated control groups; #$P<0.05$, ##$P<0.01$, ###$P<0.001$ as compared with respective SCH 58261 untreated control groups (by Newman-Keuls *post hoc* test following a one-way ANOVA).

Discussion

The main finding of the present study cements the conclusion that the stimulant effects of caffeine are derived from adenosine A_{2A} receptor blockade (Ledent *et al*., 1997; Snyder, 1997).

Caffeine is a non-selective A_1 and A_{2A} antagonist (Jacobson & van Rhee, 1997). Although it was initially suggested that A_1 receptors might be the main target for the stimulatory properties of caffeine (Snyder *et al*., 1981; Katims *et al*., 1983), the importance of adenosine A_{2A} receptors in this action

has more recently been illustrated by several behavioural and functional studies (Svenningsson *et al*., 1995; 1997; Bertorelli *et al*., 1996; Satoh *et al*., 1998). Interestingly, Svenningsson *et al*. (1995) also provided strong neurochemical evidence that transmission is specifically increased in striatopallidal neurones following acute treatment of rats with a stimulant dose of caffeine. The adenosine A_{2A} receptors are predominantly expressed in pallidal-projecting GABAergic enkephalin-containing neurones, which also express dopamine D2 receptors (Ongini & Fredholm, 1996; Johansson *et al*., 1997; Ledent *et al*., 1997). Also, at low stimulating doses in humans (Fredholm, 1995), the A_{2A} adenosine receptor was reported to be the main target of caffeine. In the present study, a wide range of pharmacologically relevant doses of caffeine (6.25–100 mg kg^{-1} i.e. 32–515 μM kg^{-1}) caused biphasic effects on locomotor activity and rearing behaviour in wild-type mice. Both horizontal and vertical components of locomotor activity were increased for moderate doses of caffeine ranging from 6.25 to 25 mg kg^{-1} (32–129 μM kg^{-1}). A 2 fold increase in locomotion was reached during the second sampling period (15–30 min) and maintained up to the end of the 1-h test. A similar pattern was observed for the less well documented effect of caffeine on rearing behaviour. A 3 fold increase in vertical activity was also reached for the three lowest doses of caffeine, during the last 45 min of the test. These data are consistent with the results obtained in previous studies in mice (Boissier & Simon, 1965; Waldeck, 1975; Kaplan *et al*., 1989; Nikodijevic *et al*., 1993) and rats (Thithapandha *et al*., 1972; Finn *et al*., 1990; Holtzman, 1991; Svenningsson *et al*., 1995). The fact that caffeine increased both the horizontal and the vertical components of locomotor activity in mice (present study) and in rats (Svenningsson *et al*., 1995) is likely to be a direct consequence of antagonism at ventral and dorsal striatum adenosine A_{2A} receptors respectively, since an activation of dopaminergic transmission in the nucleus accumbens has been linked to locomotor hyperactivity whereas the caudate-putamen plays an important role in rearing behaviour (Al-Khatib *et al*., 1995). The fact that A_{2A} receptor knockout mice did not respond to caffeine by increased locomotor activity, but rather by a dose-dependent and monophasic reduction of activity further demonstrates the role of the A_{2A} receptor in this process.

High doses of caffeine may induce dysphoria and anxiety in humans (Uhde *et al*., 1984; Griffiths & Woodson, 1988), especially in susceptible individuals (Boulenger *et al*., 1984; Charney *et al*., 1985). It has been previously suggested that administration of high doses of caffeine combined with behavioural stress induced an enhancement of the stress response as measured by sympathetic activation, pituitary-adrenal stimulation and behaviours associated with stress, both in mice (Henry & Stephens, 1980) and humans (Al Absi *et al*., 1998). In addition, caffeine has been shown to produce anxiogenic-like effects in mice, in several tests sensitive to stress-eliciting stimuli (Jain *et al*., 1995; El Yacoubi *et al*., submitted). The altered locomotor activity of rats treated with caffeine has already been shown to be environmentally determined: the drug elicited stimulatory effects in a familiar environment and suppressive effects in a novel environment (Britton & Indyk, 1990). One experiment addressed the question of whether the decrease in locomotor activity reported after administration of high doses of caffeine in mice (Boissier & Simon, 1965; Logan *et al*., 1986; Nikodijevic *et al*., 1993; this study) reflected the response of the animal to anxiogenic or stress-related stimuli or, on the other hand, is a purely locomotor phenomenon. In the present study, a high dose of caffeine (100 mg kg^{-1}) induced suppressive effects upon locomotor activity when administered to mice just before the confrontation with a novel environment but not to mice habituated to that environment (Figure 2). This finding confirms that the differing effects of caffeine in a novel versus a familiar environment (Britton & Indyk, 1990) might be related to differences in the perceived aversion of the environment and not only to the degree of ongoing activity. Interestingly, in the Svenningsson study (1995), the highest dose of caffeine (100 mg kg^{-1}) caused a significant reduction of both rearing and locomotion at the first hour post-treatment, but the rats treated with 100 mg kg^{-1} tended to be more active than vehicle-treated animals at later time-points. It is noteworthy that, in habituated mice, caffeine increased locomotion over a wide range of doses during the whole duration of the experiment, suggesting a significant increase of wakefulness at these doses. Again, there is increasing evidence suggesting that the blockade of A_{2A} receptors (Bertorelli *et al*., 1996; Satoh *et al*., 1998) plays a role in the modulation of wakefulness induced by caffeine (Schwierin *et al*., 1996).

Central effects elicited by caffeine, at pharmacologically relevant doses, are believed to be solely mediated by an interaction with A_1 or A_2 subtypes of adenosine receptors since the brain concentration attained is not high enough to elicit blockade of the adenosine 3′.5′-cyclic monophosphate (cyclic AMP)-phosphodiesterase activity (Fredholm, 1995). It was therefore of interest to compare, in the same experimental conditions, the effects of caffeine with those of selective ligands acting at A_1 receptors. Adenosine A_1 receptors are especially abundant in the cortex, hippocampus and cerebellum, but are also present in all areas of the brain, including the caudate-putamen and globus pallidus (Johansson *et al*., 1996). In the present study, the selective adenosine A_1 receptor agonist CPA (N^6-cyclopentyladenosine) generated a dose-dependent decrease in horizontal locomotor activity in CD1 male mice. It is noteworthy that CPA, over a wide range of doses, was devoid of any stimulant effects, in agreement with other studies in mice (Durcan & Morgan, 1989; Heffner *et al*., 1989; Ferré, 1997) or rats (Brockwell & Beninger, 1996; Marston *et al*., 1998). By contrast, other A_1 selective adenosine agonists R-PIA (Dunwiddie & Worth, 1982; Katims *et al*., 1983) and CCPA (Florio *et al*., 1997) have been shown to induce an increase in locomotor activity in a narrow range of low subsedative doses. Florio *et al*. (1997) suggested that this effect was mediated by an adenosine A_1 receptor, but this stimulant effect could not be antagonized by caffeine in another study (Katims *et al*., 1983). In our experimental conditions, this putative stimulant effect of adenosine A_1 agonists was not unmasked in A_{2A} receptor knockout mice receiving a low dose (0.03 mg kg^{-1} i.p.) of CPA (data not shown). Further studies using R-PIA or CCPA might help to resolve these discrepancies.

The administration of a selective A_1 receptor antagonist to A_{2A} receptor knockouts or to wild-type mice also treated with a selective A_{2A} receptor antagonist may help to better understand the role played by adenosine A_1 receptor blockade in the caffeine-mediated locomotor effects. The selective adenosine A_1 receptor antagonist DPCPX (Jacobson & Van Rhee, 1997) affected exploratory locomotor activity in CD1 mice only after administration of the highest tested dose (25 mg kg^{-1}), although the drug is well known to penetrate easily into the brain (Bisserbe *et al*., 1992; Kaplan *et al*., 1992; Marston *et al*., 1998). Previous studies have already demonstrated that DPCPX induced little changes in exploratory behaviour in mice (Griebel *et al*., 1991; Florio *et al*., 1997) or rats (Wood *et al*., 1989; Svenningsson *et al*., 1997). Whereas

there is strong evidence that blockade of adenosine A_{2A} receptors plays an important role for the stimulating action of caffeine in rodents, the role of the A_1 receptor in the behavioural depressant effects induced by caffeine in A_{2A} receptor knockout mice was previously suggested (Ledent *et al*., 1997; Snyder, 1997) but not proven. In our experimental conditions, a moderate dose of DPCPX significantly decreased locomotor activity in the A_{2A} receptor knockout mice without altering the behaviour of wild-type mice. This finding strongly supports the hypothesis put forward by Svenningsson *et al.* (1995; 1997), that behavioural depression and increased expression of immediate early genes elicited by high doses of caffeine in rat caudate-putamen are mediated by the blockade of A_1 receptors. Interestingly, a significant decrease in rearing behaviour was observed both in rats receiving DPCPX (Svenningsson *et al*., 1997) and in A_{2A} receptor knockout mice.

Finally, DPCPX was used in CD1 mice in combination with the novel A_2 selective antagonist SCH 58261. It appears that activation of either A_1 or A_{2A} receptors can lead to locomotor depression, and it was also shown previously that a synergism between these depressant effects may occur when agonists selective for each receptor subtype are combined (Nikodijevic *et al*., 1991). A similar synergism between an A_1 antagonist (DPCPX) and an A_{2A} antagonist (8-3(chlorostyryl)caffeine) has been found in a previous study (Jacobson *et al*., 1993). However, even at the highest tested dose, the chosen A_{2A} antagonist did not display clear stimulant locomotor effects in that study. More recently, three other selective A_{2A} antagonists became available: KF 17837, an 8-styryl xanthine derivative, and two non-xanthine molecules, SCH 58261 and ZM 241385 (Ongini & Fredholm, 1996). In the present study, the acute administration of SCH 58261, at moderate doses, caused a weak but significant stimulant effect on locomotor activity in mice, in agreement with previous findings (Svenningsson *et al*., 1997; El Yacoubi *et al*., 1998). However, animals receiving both DPCPX and SCH 58261 were less active than animals treated with SCH 58261 alone; resulting in an interaction between the two drugs. Therefore, the present results do not substantiate the notion that adenosine A_1 receptor blockade synergizes with adenosine A_{2A} receptor blockade to mediate the locomotor activating properties of caffeine.

A note of caution should be made before concluding. As previously mentioned by several authors (Snyder *et al*., 1981; Svenningsson *et al*., 1997), locomotor stimulation by caffeine may vary according to the methodological approaches used to record the behaviour of the animals. The environment, i.e. the stress of novelty, probably plays an important role in the observed effects. Indeed, the behavioural profiles of SCH 58261 in the two experimental paradigms used in this study differ with that of caffeine (El Yacoubi *et al*., submitted for publication), and this selective A_{2A} antagonist does not share the anxiogenic properties of the latter drug (El Yacoubi *et al*., in press). When trying to reconcile data from different experiments these findings should be kept in mind.

In summary, the present results show that the caffeine-induced increase in locomotor activity is caused by the blockade of the adenosine A_{2A} receptor. Additional evidence is provided for the notion that the depressant effects observed after acute administration of high doses of caffeine are mainly caused by blockade of the adenosine A_1 receptor. These inhibitory effects upon exploration could also be linked to the perceived aversion of the environment. Inter-individual differences in neuropharmacological responsiveness to caffeine, likely linked to different ratios in the cerebral density of A_1 and A_{2A} receptors, may actually contribute to the wide variation of manifestations elicited by the consumption of caffeine-containing beverages among individuals (Charney *et al*., 1985; Kendler & Prescott, 1999).

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