British Journal of Pharmacology (1995) 116, 2127-2133

Anxiolytic activity of adenosine receptor activation in mice

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1 Purine analogues have been examined for anxiolytic- and anxiogenic-like activity in mice, by use of the elevated plus-maze.

2 The selective A_1 receptor agonist, N^6-cyclopentyladenosine (CPA) had marked anxiolytic-like activity at 10 and 50 $\mu g\ kg^{-1}$, with no effect on locomotor performance at these doses.

3 The A_1 selective adenosine receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (CPX) had no significant effect on anxiety-related measures or locomotor behaviour, but blocked the anxiolytic-like activity of CPA. The hydrophilic xanthine, 8-(*p*-sulphophenyl) theophylline did not prevent anxiolysis by CPA.

4 Caffeine had anxiogenic-like activity at 30 $mg\ kg^{-1}$ which was prevented by CPA at 50 $\mu g\ kg^{-1}$.

5 The A_2 receptor agonist, N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine (DPMA) had no effect on anxiety behaviour but depressed locomotor activity at the highest dose tested of 1 $mg\ kg^{-1}$. The A_2 receptor antagonist, 1,3-dimethyl-1-propargylxanthine (DMPX) had no effect on anxiety-related measures or locomotion and did not modify the anxiolytic-like activity of CPA.

6 Administration of DPMA in combination with anxiolytic doses of CPA prevented the anxiolytic-like activity of the latter.

7 The results suggest that the selective activation of central A_1 adenosine receptors induces anxiolytic-like behaviour, while the activation of A_2 sites causes locomotor depression and reduces the effects of A_1 receptor activation. The absence of any effect of CPX alone suggests that the receptors involved in modulating behaviour in the elevated plus-maze in mice are not activated tonically by endogenous adenosine.

Keywords: Adenosine; purines; anxiety; plus-maze; xanthines

Introduction

Adenosine is able to modulate neuronal activity in the central nervous system by acting at presynaptic and postsynaptic sites (see Stone & Simmonds, 1991, for review). The presynaptic sites may be a combination of A_1 and A_2 receptors, activation of the former generally inhibiting the release of neurotransmitters such as glutamate (Clark & Dar, 1989; Corradetti *et al.*, 1984; Fastbom & Fredholm, 1985; Prince & Stevens, 1992), acetylcholine (Spignoli *et al.*, 1984), dopamine (Michaelis *et al.*, 1979; Zetterstrom & Fillenz, 1990), 5-hydroxytryptamine (Feuerstein *et al.*, 1985) and noradrenaline (Jonzon & Fredholm, 1984). The A_2 receptors tend to increase the release of some of these transmitters (Spignoli *et al.*, 1984; Correiadesa *et al.*, 1991; Kirkpatrick & Richardson, 1993). Postsynaptically, adenosine and its analogues can increase potassium (Haas & Greene, 1984; Trussel & Jackson, 1985) and chloride conductances (Mager *et al.*, 1990; Akhondzadeh & Stone, 1994) and can modulate neuronal sensitivity to transmitters such as acetylcholine (Brooks & Stone, 1988) and dopamine (Ferre *et al.*, 1991).

It is presumably as a result of these properties that adenosine analogues exhibit a range of behavioural effects, which include sedation (Barraco *et al.*, 1983; Dunwiddie & Worth, 1982; Snyder *et al.*, 1981), anticonvulsant activity (Barraco *et al.*, 1984; Dunwiddie & Worth, 1982), antinocisponsive effects (Ahlijanian & Takemori, 1985; Holmgren *et al.*, 1983; Yarbrough & McGuffin-Clineschmidt, 1981), suppression of operant responding (Coffin & Spealman, 1985; 1987) and conditioned avoidance responding (Martin *et al.*, 1993) inhibition of aggression (Palmour *et al.*, 1989) and changes of cognitive function (Winsky & Harvey, 1986).

Studies of the possible effects of purines on anxiety-related behaviour, however, have been inconclusive, with several studies indicating anxiogenic-like activity. Commissaris *et al.* (1990) reported that R-N6-phenylisopropyladenosine (R-PIA) or 5'N-ethylcarboxamide adenosine (NECA) did not yield any anticonflict activity in a punished responding paradigm, while the results of Haraguchi & Kuribara (1991) indicate that a reliable suppression of punished responding could be obtained, but only at doses of 0.1 and 0.3 $mg\ kg^{-1}$ which also depressed non-punished responding. R-PIA and NECA have both been claimed to facilitate shock avoidance at some doses (Coffin & Spealman, 1985), while low doses of R-PIA could inhibit the increase of punished responding and other behaviours produced by diazepam (Coffin & Spealman, 1985; Haraguchi & Kuribara, 1991). In the social interaction test 2-chloroadenosine was reported to have no effect on behaviour (Baldwin & File, 1989), although it is unlikely that this agent would cross the blood-brain barrier in significant amounts.

The purine antagonist caffeine has also been shown to have anxiogenic-like activity in animals and human subjects (Baldwin & File, 1989; Charney *et al.*, 1985; Loke *et al.*, 1985; Unde *et al.*, 1984). Caffeine, however, is non-selective at A_1 and A_2 purinoceptors. The A_1 receptor-selective antagonist, 1,3-dipropyl-8-cyclopentylxanthine (CPX) was reported by Griebel *et al.* (1991) to have no effect on anxiety behaviour, whereas the non-xanthine antagonist 9-chloro-2-(2-furyl)-5,6-dihydro-[1,2,4]triazolo[1,5-c]quinazolin-5-imine (CGS15943A), with antagonist activity at A_1 and A_2 receptors, did show anxiogenic properties.

The present study was designed to investigate the effect of selective A_1 and A_2 receptor agonists and antagonists on mouse anxiety behaviour in the elevated plus-maze.

Methods

Animals

Male ICR or MF1 mice weighing 25-30 g were housed in groups of ten under a 12 h light: 12 h dark cycle, with free access to food and water.

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Treatment

Animals were injected intraperitoneally with drugs in a volume of 10 ml kg^{-1} (approx. 250 μl per mouse) and were tested in the plus-maze 30 min later. When combinations of agonist and antagonist agents were employed, the antagonist was administered 5 min before the agonist. An exception to this was caffeine, which preliminary experiments revealed had anxiogenic activity 15 but not 30 min after administration. Caffeine was therefore routinely injected 15 min before maze testing, whatever the combination being used.

Apparatus

The elevated plus-maze consisted of two open arms, 21 by 8 cm, and two enclosed (walled) arms 21 × 8 × 12 cm arranged in a cross formation. The maze was fixed at a height of 15 cm. above its base.

Procedure

Mice were placed individually on the central 'neutral' square between the four arms of the plus-maze. Entry into an arm was noted only when all four paws had crossed out of the central square into an arm area. Two scores were made by a trained observer over a 5 min period: (i) the total number of entries made into all arms [TE] and (ii) the time spent on the open arms. The latter was later used to calculate the percentage of time spent on the open arms [%OT].

Statistics

Results were analysed by analysis of variance (ANOVA) followed, where allowed, by a Newman-Keuls test for multiple comparisons where appropriate. Significance was defined as $P<0.05$.

Materials

N^6-cyclopentyladenosine (CPA), 1,3-dipropyl-8-cyclopentylxanthine (CPX), caffeine, 8-(*p*-sulphophenyl)theophylline (8PST), DMPA and DPMX were purchased from Research Biochemicals. Wherever possible drugs were injected in 0.9% saline, which was then used for the respective control groups. CPX was dissolved in 95% ethanol and DPMA and DMPX were dissolved in methanol to produce stock solutions which could be diluted with saline. Control animals were treated with the same dilution of methanol or ethanol. Diazepam was used as diazepam for injection (10 mg in 2 ml; Pharma- Hameln, Germany).

Results

A preliminary experiment was performed to confirm that both anxiolytic and anxiogenic effects could be demonstrated in the plus-maze. Groups of 10 mice were treated with 0.9% saline, caffeine 30 mg kg^{-1} 15 min before testing or diazepam 2 mg kg^{-1} 30 min before testing. Caffeine reduced %OT scores by approximately 30% ($P<0.05$) whereas diazepam increased these scores by almost 100% ($P<0.05$) (Figure 1). Neither drug induced any change of total arm entries.

Adenosine receptor agonists

Cyclopentyladenosine Groups of 20 mice were injected with N^6-cyclopentyladenosine (CPA) in doses of 10, 50, 100, 200 or 250 μg kg^{-1} and tested 30 min later. At the lower two doses, significant changes were detected in %OT compared with controls, though no changes were seen in TE (Figure 2). The three higher doses had no effect on %OT, but the highest dose of 250 μg kg^{-1} had a general depressant action on the animals' behaviour, reducing TE below the level of control mice (Figure 2).

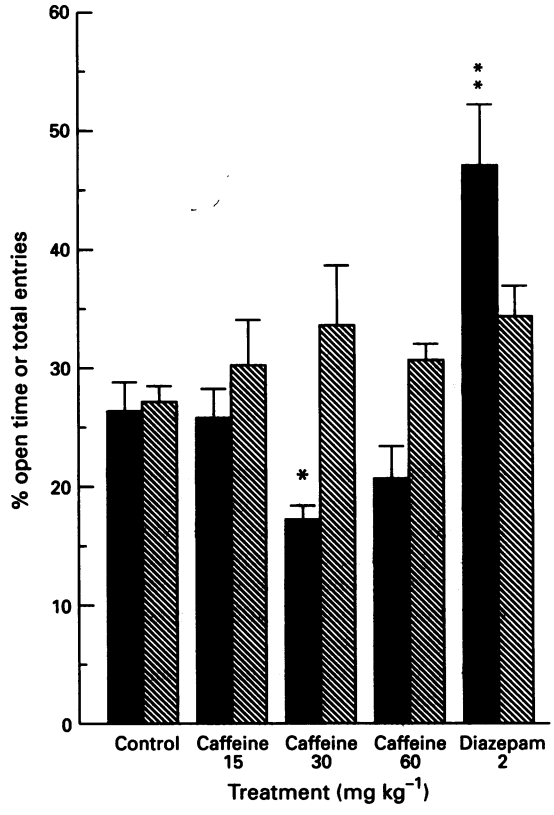

Figure 1 Histogram of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with vehicle (control), caffeine 15, 30 or 60 mg kg^{-1} or diazepam 2 mg kg^{-1}. *$P<0.05$; **$P<0.01$ relative to vehicle controls.

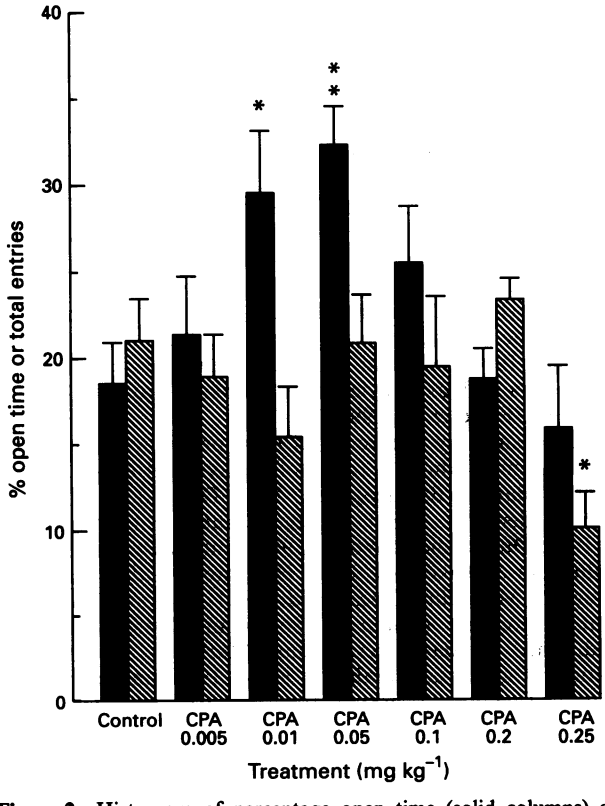

Figure 2 Histogram of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with vehicle (control) or CPA 0.005, 0.01, 0.05, 0.1, 0.2 or 0.25 mg kg^{-1}. *$P<0.05$; **$P<0.01$ relative to vehicle controls.

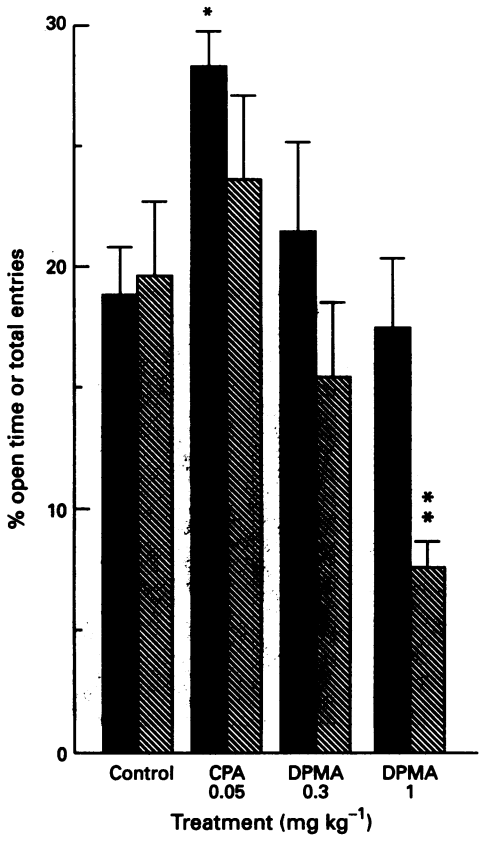

Figure 3 Histogram of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with vehicle (control), CPA 0.05 mg kg^{-1}, DPMA 0.3 or 1 mg kg^{-1}. *$P<0.05$; **$P<0.01$ relative to vehicle controls.

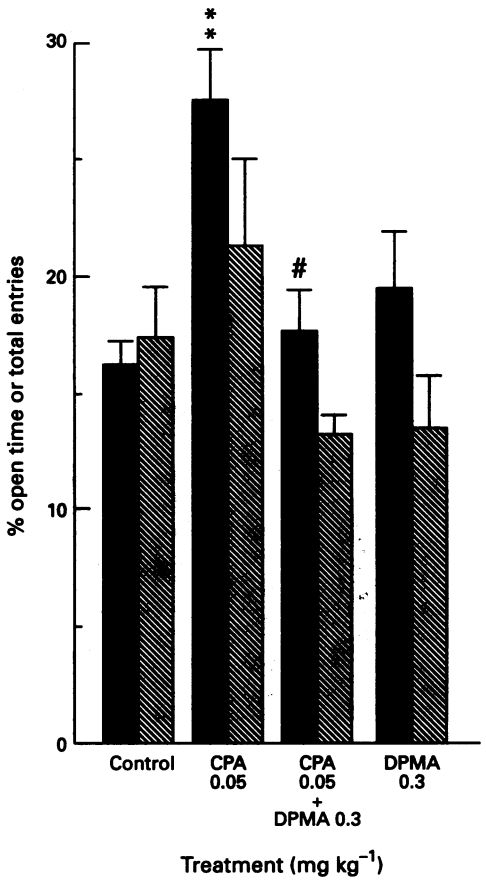

Figure 4 Histogram of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with vehicle (control), CPA 0.05 mg kg^{-1}, DPMA 0.3 mg kg^{-1} or a combination of CPA 0.05 with DPMA 0.3 kg kg^{-1}. *$P<0.05$; **$P<0.01$ relative to vehicle controls; #$P<0.05$ relative to CPA alone.

DPMA The administration of DPMA at 0.3 mg kg^{-1} had no significant effect upon %OT or TE. However, at a higher dose of 1 mg kg^{-1} there was a significant reduction of TE (Figure 3).

Combinations of agonists The combination of 0.05 mg kg^{-1} CPA and 0.3 mg kg^{-1} DPMA yielded behaviour scores which were not different from vehicle controls but which were significantly different from the effect of CPA alone (Figure 4).

Adenosine receptor antagonists

CPX The A_1 receptor selective antagonist, 1,3-dipropyl-8-cyclopentylxanthine was injected at 0.05 or 0.5 mg kg^{-1} and the mice tested 30 min later (Figure 5). There was no statistically significant effect on either %OT or TE at either of the doses tested. In the same experiment, caffeine still showed anxiogenic-like activity.

DPMX When administered alone at 1 mg kg^{-1}, DPMX induced no change in %OT. While there was an increase in the mean value of TE in these mice, the change was not statistically significant (Figure 5).

Combinations of agonists and antagonists

CPA and CPX CPA was administered at the anxiolytic dose of 50 μg kg^{-1} 5 min after CPX at 50 μg kg^{-1}, a dose which by itself had no observable effect on behaviour. This combination resulted in a blockade of the anxiolytic-like effect of CPA (Figure 6).

CPA and DPMX At 1 mg kg^{-1} DPMX was not able to prevent the anxiolytic-like activity of CPA given at 0.05 mg kg^{-1} (Figure 7).

CPA and caffeine The administration of a low dose of CPA (5 μg kg^{-1}) which alone had no effect on behaviour, together with caffeine at 30 mg kg^{-1} raised %OT to a level which was not significantly different from controls. However, this %OT was still not significantly different from CPA alone (Figure 8a). With an anxiolytic dose of 50 μg kg^{-1} CPA, the anxiogenic-like effect of caffeine and the anxiolytic-like activity of CPA were both lost, the combination yielding %OT scores which were not significantly different from controls, but which were different from CPA alone. This combination of drugs also yielded a significant increase of total entries compared with control animals, but not compared with CPA or caffeine alone (Figure 8b).

CPA and 8-(p-sulphophenyl)theophylline When CPA at 0.05 mg kg^{-1} was injected together with the hydrophilic xanthine, 8PST at 10 mg kg^{-1}, there was no reduction of the anxiolytic-like activity of the agonist (Figure 9).

Discussion

The elevated plus-maze has been validated by several previous groups, using a wide range of agents known to exhibit anxiolytic-like or anxiogenic-like activity in more sophisticated animal models or in human subjects (Pellow & File 1986; Lister 1987; Handley & McBlane, 1993; but see Rodgers & Cole, 1994). The plus-maze is, in effect, a mild anti-conflict paradigm with self-exposure to a novel, potentially dangerous environment as the deterrent stimulus. We have confirmed that in the experimental apparatus, environmental conditions and mouse strains employed in the present work, the technique reveals

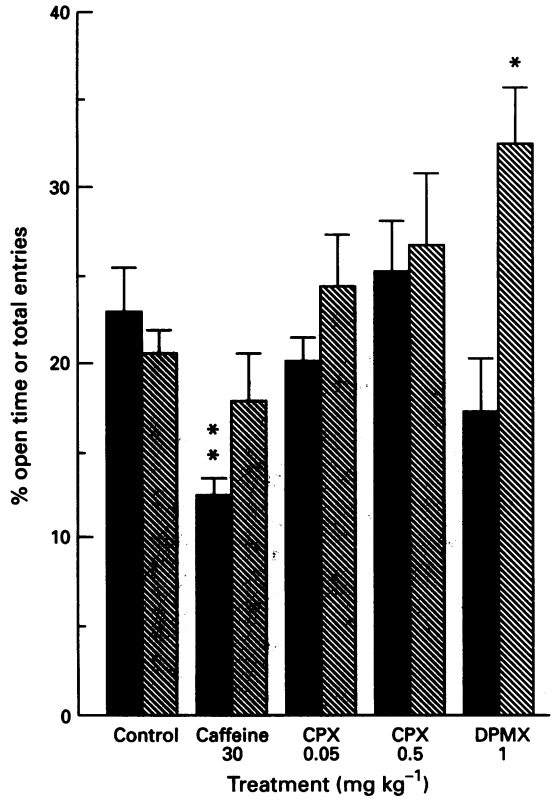

Figure 5 Histogram of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with vehicle (control), caffeine 30 mg kg^{-1}, CPX 0.05 or 0.5 mg kg^{-1} or DPMX 1 mg kg^{-1}. *$P<0.05$; **$P<0.01$ relative to vehicle controls.

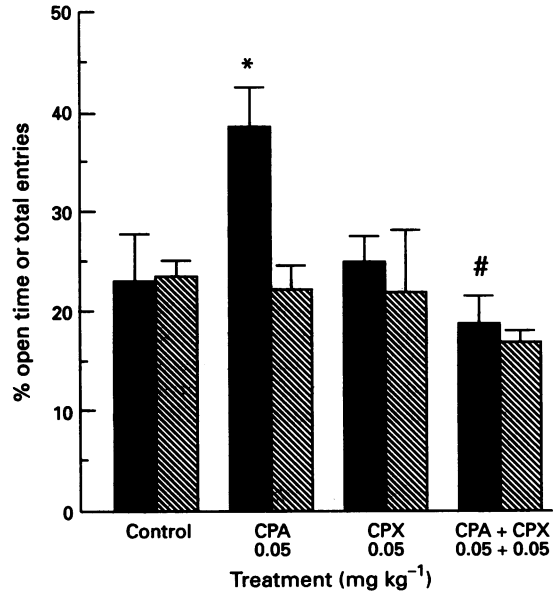

Figure 6 Histogram of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with vehicle (control), CPA 0.05 mg kg^{-1}, CPX 0.05 mg kg^{-1} or a combination. *$P<0.05$; **$P<0.01$. #$P<0.05$ relative to CPA alone.

both anxiolytic activity of diazepam and a mild anxiogenic profile of caffeine. Neither drug produced any change in the total number of arm entries made, thus indicating a lack of any overall sedative or motor depressant activity at the doses used.

CPA is an agonist with high selectivity for the A_1 subtype of adenosine receptor, its K_d at A_1 receptors (0.5 nM) being almost 2500 times less than its K_d at A_2 sites (Williams *et al.*,

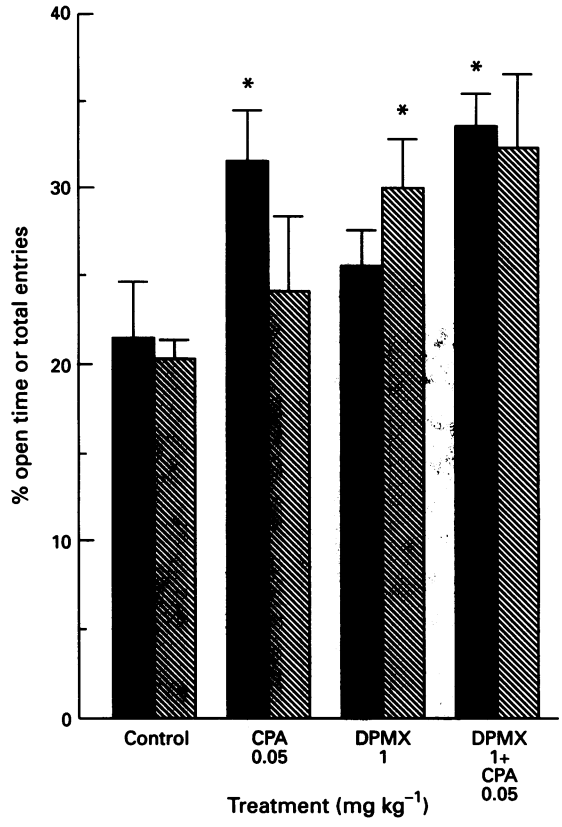

Figure 7 Histogram of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with vehicle (control), CPA 0.05 mg kg^{-1}, DPMX 1 mg kg^{-1} and a combination of DPMX with CPA 0.05 mg kg^{-1}. *$P<0.05$; **$P<0.01$. The results with CPA + DMPX are not significantly different from CPA alone.

1986). Administration of CPA proved effective in the plus-maze over a narrow dose range, 50 μg kg^{-1} showing a very marked anxiolytic-like profile without sedation, but 250 μg kg^{-1} producing a clear depressant effect on locomotor activity.

Although the A_1 receptor selective antagonist CPX (A_1: A_2 K_d ratio of 1100; Bruns *et al.*, 1987; Lohse *et al.*, 1987) had no effect alone on behaviour, it did prove able to prevent the anxiolytic-like activity of 50 μg kg^{-1} CPA. This would strongly support the view that the anxiolytic-like activity of CPA resulted from the selective stimulation of A_1 receptors at this dose. Whereas CPX has been demonstrated to cross the blood-brain barrier (Baumgold *et al.*, 1992), the sulphonated xanthine, 8PST, is unable to do so. The failure of 8PST to afford any blockade of CPA thus presents a strong argument that the A_1 adenosine receptors involved in the anxiolytic activity are confined to the central nervous system.

A_1 receptors are widely distributed throughout the CNS, and occur both on presynaptic terminals, where they mediate a potent inhibition of the release of a variety of neurotransmitters including glutamate, acetylcholine, 5-hydroxytryptamine (5-HT) and some peptides, and postsynaptically where they can suppress neuronal activity by opening potassium channels (see Introduction). Since antagonists acting at glutamate and 5-HT receptors are known to have anxiolytic properties, it may be an inhibition of release of these endogenous agonists which is responsible for A_1 receptor-mediated anxiolysis.

One of the most intriguing findings was that administration of the A_2 receptor agonist, DPMA, together with CPA prevented the anxiolytic-like activity of the latter. This might suggest that activation of A_2 receptors is able to suppress the effects of activating A_1 receptors. A similar inhibition by A_2

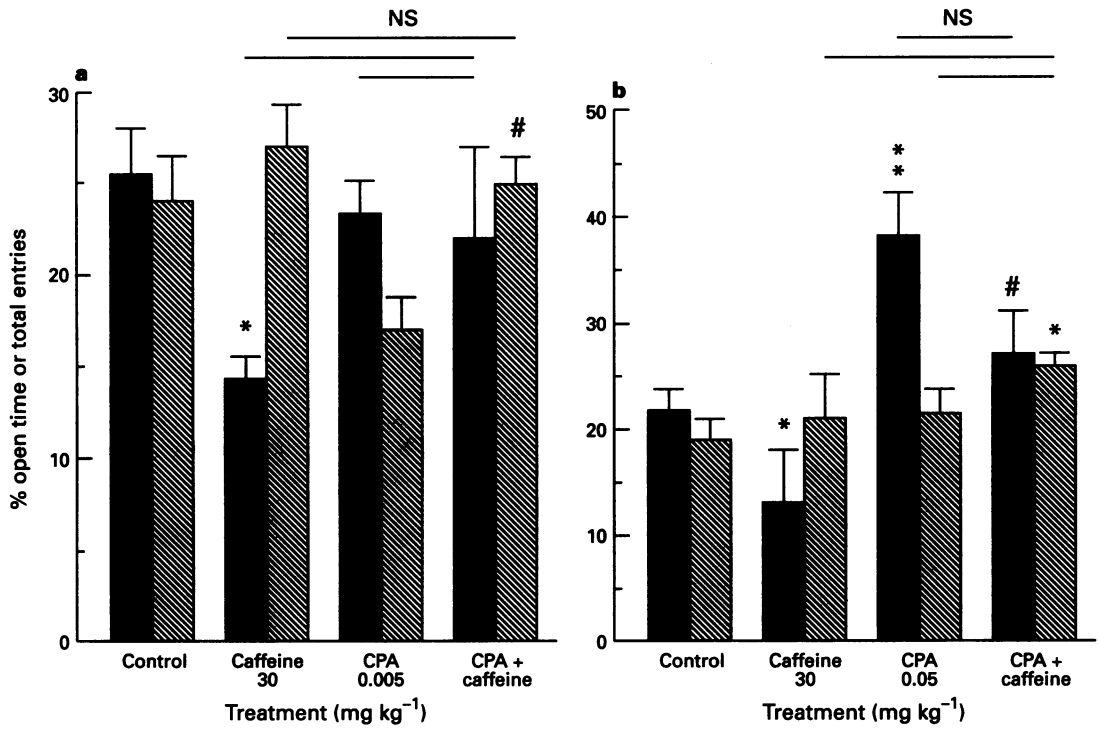

Figure 8 Histograms of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with (a) vehicle (control), caffeine 30 mg kg^{-1}, CPA 0.005 mg kg^{-1} or a combination; (b) vehicle (control), caffeine 30 mg kg^{-1}, CPA 0.05 mg kg^{-1} or a combination. $^{*}P<0.05$; $^{**}P<0.01$. In (a) $^{\#}P<0.05$ relative to CPA alone; in (b) $^{\#}P<0.05$ relative to caffeine alone. Other comparisons indicated by the horizontal lines are non-significant.

receptors of, for example, dopamine D_2 receptors has been fully characterized by Ferre *et al.* (1991). This same mechanism, A_2 receptor suppression of A_1 receptor function, has recently been proposed to explain the ability of low, but not high doses of adenosine receptor agonists to suppress amino acid release, and the ability of an A_2 receptor antagonist to inhibit release (Simpson *et al.*, 1992). It has also been invoked to explain the interactions of adenosine receptor agonists on catalepsy observed by Zarrindast *et al.* (1993).

It is interesting to note the lack of effect of CPX alone. A similar result was obtained by Griebel *et al.* (1991) using the same compound in a light/dark choice paradigm, and it implies an absence of any tonic activity of endogenous adenosine at the receptors involved in anxiolysis. This is important because it raises the problem of explaining the anxiogenic-like profile of caffeine.

When a behaviourally ineffective dose of CPA was administered together with caffeine at 30 mg kg^{-1} the anxiogenic-like action of the latter was prevented. A similar interaction was observed with caffeine and CPA at 50 μg kg^{-1}, both the respective anxiogenic and anxiolytic activities being lost. It is difficult with this latter combination to exclude a purely functional, rather than receptor-mediated, antagonism, but one explanation of this interaction may be that the anxiogenic-like activity of caffeine is due to the blockade of A_1 adenosine receptors at which endogenous adenosine is tonically active. This might imply that they are a different set of A_1 receptors from those blocked by CPX, which is not anxiogenic.

A second explanation may be that the anxiogenic-like properties of caffeine are due to the simultaneous blockade of A_1 and A_2 receptors, since caffeine has virtually no ability to distinguish between these sites in the CNS (K_i values of 29 and 48 μM respectively; Bruns *et al.*, 1987). The occurrence of such an A_2 blockade is supported by the result of combining caffeine with CPA at 50 μg kg^{-1}, the dose concluded above to activate A_1 receptors. This combination yielded a significant increase of total entries. Since activation of A_2 receptors has been linked with a depression of locomotor activity (Barraco *et al.*, 1983; 1993; Durcan & Morgan 1989; Jacobson *et al.*, 1991; Janusz & Berman 1993), this result is entirely consistent with an ability of caffeine to block A_2 receptors tonically activated by endogenous adenosine.

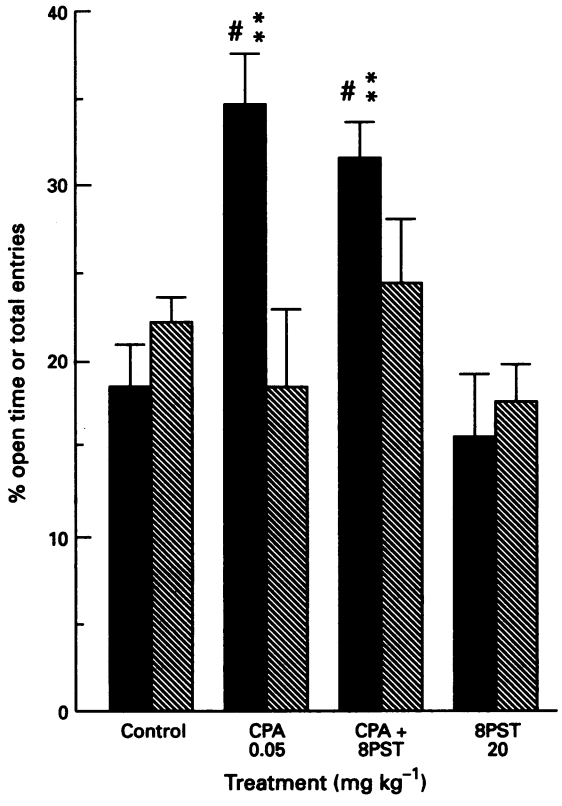

Figure 9 Histogram of percentage open time (solid columns) and total arm entries (hatched columns) for mice treated with vehicle (control), CPA 0.05 mg kg^{-1}, 8PST 20 mg kg^{-1} or CPA in combination with 8PST 20 mg kg^{-1}. $^{*}P<0.05$; $^{**}P<0.01$ relative to vehicle controls. $^{\#}P<0.05$ relative to 8PST alone.

This explanation of the anxiogenic-like activity of caffeine, that simultaneous A_1 and A_2 receptor blockade is necessary for anxiogenesis, is in accord with the finding of Griebel *et al.* (1991) that the non-xanthine antagonist, CGS15943A, which also possesses the ability to block both A_1 and A_2 receptors, also showed anxiogenic activity. The results are also interesting in view of the work of Kaplan *et al.* (1992) who attempted to correlate behavioural stimulation by caffeine with receptor occupancy. They concluded that, although caffeine did occupy CNS A_1 receptors at behavioural stimulant doses, the results could not be accounted for by A_1 receptor blockade alone. Their measurement of brain caffeine levels would indicate that the dose of caffeine used in the present work would reach around 50 μM, a concentration similar to the K_i values at A_1 and A_2 receptors quoted above.

The specific neuronal mechanisms by which adenosine receptor ligands mediate their effects on animal behavior remain uncertain. The A_2 receptors are known to be localized chiefly within the basal ganglia where they have been shown to modulate dopaminergic function by interfering with presynaptic receptors and the release of dopamine, and with postsynaptic receptors (Myers & Pugsley 1986; Ferre *et al.*, 1991). It is this siting of the A_2 receptors in basal ganglia which presumably accounts for their potent depressant effect upon locomotor activity (Durcan & Morgan, 1989; Nikodijevic *et al.*, 1990; Griebel *et al.*, 1991).

In summary the results presented here strongly suggest that activation of A_1 adenosine receptors within the CNS can produce anxiolytic behaviour, a finding which raises the possibility of developing CNS selective purine receptor agonists as novel anxiolytic drugs. The activation of A_2 receptors does not have effects on plus-maze behaviour, but suppresses the effects of activating A_1 receptors. The anxiogenic activity of caffeine, however, cannot be simply explained on the basis of a blockade of A_1 receptors, since CPX is not anxiogenic. The effects of caffeine may be a consequence of its non-selective blockade of both A_1 and A_2 receptors.

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(Received March 15, 1995
Revised May 25, 1995
Accepted June 15, 1995)