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Comparison of the behavioural effects of an adenosine A_1/A_2-receptor antagonist, CGS 15943A, and an A_1-selective antagonist, DPCPX

Guy Griebel[1], Martine Saffroy-Spittler[1], René Misslin[1], Delphine Remmy[2], Elise Vogel[1], and Jean-Jacques Bourguignon[2]

[1] Laboratoire de Psychophysiologie, 7, rue de l'Université, F-67000 Strasbourg, France
[2] Département de Pharmacochimie Moléculaire, Centre de Neurochimie, 5, rue Blaise Pascal, F-67084 Strasbourg Cedex, France

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Abstract. CGS 15943A is the first reported nonxanthine adenosine antagonist and it shows high affinity towards A_1 and A_2 receptors. The present data show that CGS 15943A increased in a dose-dependent manner locomotor activity of mice confronted with a free exploratory test without markedly modifying rears or, at low or medium doses, novelty seeking responses. In the light/dark choice procedure, which is especially appropriate for revealing anxiolytic and anxiogenic drug-effects, CGS 15943A decreased the time spent by mice in the lit box and increased the number of transitions. By contrast, the highly selective adenosine A_1 receptor, DPCPX, did not significantly modify the behavior of mice except at high doses, which decreased it in the free exploratory test. It is suggested that the present findings confirm the hypothesis that the behavioral effects of adenosine antagonists are linked to their actions at adenosine A_2 receptors.

Key words: CGS 15943A – DPCPX – Adenosine antagonists A_1 and A_2 – Locomotion – Anxiety

The finding that methylxanthines such as caffeine and theophylline were competitive pharmacological antagonists of the adenosine-stimulated formation of cAMP in brain slices (Sattin and Rall 1970) suggested that adenosine is an important regulator of central neurotransmission. There is available evidence that adenosine exerts a tonic inhibitory action referred to as "purinergic tone", since it has been found to depress cellular activity, which appears to be partly due to an inhibition of transmitter release (Fredholm and Hedqvist 1980; Dunwiddie 1985). Theophylline and caffeine are well known to increase locomotion in mice (Snyder et al. 1981; Logan et al. 1986; Seale et al. 1986) while analogs of adenosine decrease it (Barraco et al. 1983; Durcan and Morgan 1989; Heffner et al. 1989; Nikodijevic et al. 1990). Furthermore, caffeine has been found clinically to increase anxiety in healthy volunteers (Uhde et al. 1984; Charney et al. 1985; Loke et al. 1985; Shanahan and Hughes 1986), while in rats this drug acts in the opposite direction to the effects of anxiolytic compounds (File et al. 1988; Baldwin and File 1989) and in mice it increases the stress-induced hormonal and pathophysiologic changes (Henry and Stephens 1980). The behavioural effects of methylxanthines appear to be mediated at two non-specific subtypes of extracellular recognition sites termed A_1 and A_2. Although the central stimulant action of these drugs in mice has been found to correlate with their capacity to compete with radioligands binding to adenosine A_1 receptors (Snyder et al. 1981), more recent data suggest that the A_2 receptor subtype may be responsible for these effects (Seale et al. 1986; Spealman and Coffin 1986; Durcan and Morgan 1989; Heffner et al. 1989; Nikodijevic et al. 1990).

The present report describes the comparative effects of the first reported nonxanthine triazoloquinazoline adenosine A_1 and A_2 antagonist, 9-chloro-2-(2-furyl)-5,6-dihydro [1, 2, 4] triazolo [1,5-c]quinazolin-5-imine (CGS15943A) (Ghai et al. 1987) and the highly selective, competitive A_1-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (Lohse et al. 1987) on the behaviour of mice confronted with a free exploratory test situation and an unconditioned light/dark conflict procedure. The first experiment was undertaken in order to evaluate the drug-effects on the responses of mice towards novelty and on their locomotor activity. The second experiment was concerned with potential "anxiogenic" properties of these drugs. Finally, insofar as CGS 15943A has been reported as a non-selective adenosine receptor antagonist while DPCPX is a highly selective A_1 antagonist, this study may also contribute to show whether the behavioral modifications induced by these drugs were related to one or the other adenosine subtype receptor.

Offprint requests to: R. Misslin

Materials and methods

Animals

Male Swiss albino mice from "Centre d'Elevage R. Janvier" (France), 12 weeks of age at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a constant supply of food pellets and water, and kept on a 12/12 h light/dark cycle with lights on at 1 a.m. in order to observe animals in their high activity period, that is when lights are off. In all experimental procedures, each mouse was only tested once.

Drugs

CGS 15943A and DPCPX were dissolved in saline with a drop of Tween 80 and administered intraperitoneally, 30 min before testing, in concentrations giving an injection volume of 10 ml/kg body wt.

Statistical analysis

Statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance and a Dunnett's (equality of variances are assumed to be equal) or Bonferroni's (variances are not assumed to be equal) a posteriori *t*-test.

Experiment 1

Apparatus. The apparatus consisted of a polyvinylchloride box ($30 \times 20 \times 20$ cm) covered with plexiglas and subdivided into six equal square exploratory units, which were all interconnected by small doors. It could be divided in half lengthwise by closing three temporary partitions. The apparatus was kept on a stand in the mouse room. The experimenter stood next to the box always at the same place.

Procedure. Approximately 24 h before testing, each subject was placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. Only the floor of the familiar half was covered with sawdust and the animal was given unlimited access to food and water. Next day, the subject was exposed to both familar and novel compartments by removal of the temporary partitions. It was then observed, in red light, for 10 min. The time spent in the novel half (novelty preference), the number of units entered (locomotion) and the number of rears made by the animals was recorded.

Mice were randomly allocated into six groups receiving vehicle ($n=20$) or CGS 15943A (1, 2, 4, 8, 16 mg/kg; $n=10$) and into seven groups receiving vehicle ($n=24$) or DPCPX (0.18, 0.37, 0.75, 1.5, 3, 6 mg/kg; $n=10$).

Experiment 2

Apparatus. The apparatus consisted of two polyvinylchloride boxes ($20 \times 20 \times 14$ cm) covered with plexiglas. One of these boxes was darkened with cardboard. A light from a 100 W desk lamp, 25 cm above the other box provided the only room illumination. An opaque plastic tunnel ($5 \times 7 \times 10$ cm) separated the dark box from the lit one. During observation, the experimenter sat at the same place, next to the apparatus.

Procedure. The subjects were individually tested in 5-min sessions in the apparatus described above. The floor of the boxes was cleaned between test sessions. Testing was performed between 2 p.m. and 4 p.m. Mice were placed in the lit box to start the test session. The amount of time spent by mice in the lit box (TLB) and the number of transitions through the tunnel were recorded over a 5-min period, after the first entry in the dark box. A mouse whose four paws were in the new box was considered as having changed boxes.

Mice were randomly divided into four groups receiving vehicle ($n=30$) or CGS 15943A (1, 2, 4, 8 mg/kg; $n=15$) into five groups receiving vehicle ($n=15$) or DPCPX (0.5, 1, 1.5, 2 mg/kg; $n=15$).

Results

Experiment 1

In mice treated with CGS 15943A, ANOVA revealed significant group differences for novelty preference [$F(5,64)=5.36$; $P<0.001$)] and for locomotion

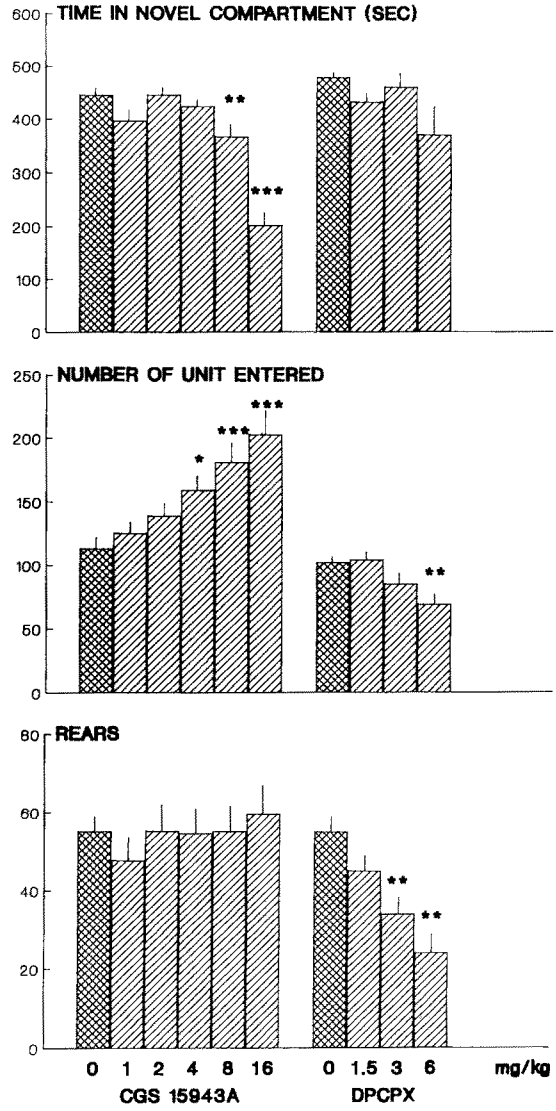

Fig. 1. Effects of CGS 15943A and DPCPX on the time (means ± SEM) spent by mice in the novel compartment (novelty preference), the number of unit entered (locomotion) and the number of rears in the 10-min free exploratory test. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

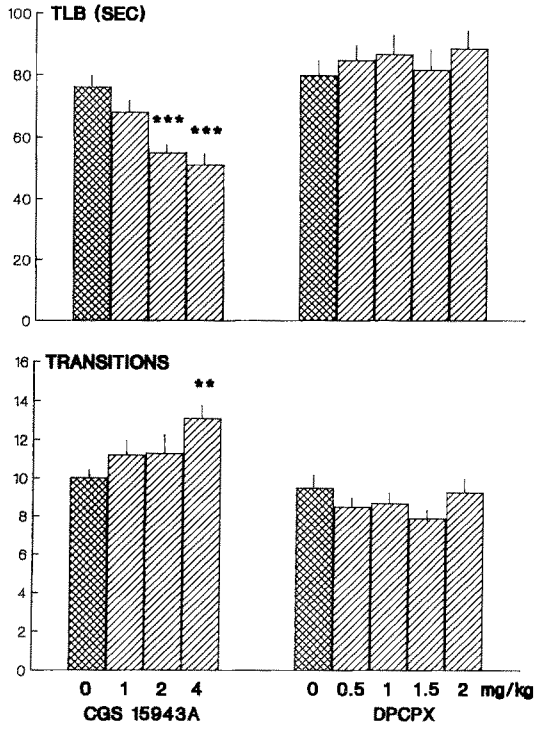

Fig. 2. Effects of CGS 15943A and DPCPX on the time spent by mice in the lit box (TLB) (means ± SEM) as well as on the number of transitions between the two boxes. ** $P<0.01$; *** $P<0.001$

[$F(5,64)=9.18$; $P<0.001$], but not for number of rears [$F(5,64)=0.83$]. Figure 1 shows that this drug significantly decreased novelty preference at the two highest doses (8 and 16 mg/kg) (Dunnett's t-test) while it induced a dose-dependent increase in locomotion (Dunnett's t-test).

In mice treated with DPCPX, ANOVA revealed significant group differences for novelty preference [$F(6,77)=2.21$; $P<0.05$], for locomotion [$F(6,77)=3.16$; $P<0.008$] and for rears [$F(6,77)=5.62$; $P<0.001$]. Figure 1 shows that the drug did not significantly modify novelty preference at any dose (Bonferroni's t-test) although the ANOVA groups main effect was significant, but it significantly decreased locomotion at the highest dose (6 mg/kg) as well as number of rears at 3 and 6 mg/kg (Dunnett's t-test).

Experiment 2

In mice treated with CGS 15943A, ANOVA revealed group differences for TLB [$F(4,85)=7$; $P<0.001$] and for number of transitions [$F(4,85)=3.91$; $P<0.006$]. Figure 2 shows that the drug significantly decreased TLB at 2 and 4 mg/kg (Bonferroni's t-test) and significantly increased number of transitions at 4 and 8 mg/kg (Dunnett's t-test).

In mice treated with DPCPX, ANOVA revealed no significant group differences either for TLB [$F(4,70)=1.48$] or four number of transitions [$F(4,70)=1.17$] (Fig. 2).

Discussion

The results of the present study demonstrate that administration of increasing doses of CGS 15943A induced a clear-cut dose-dependent increase of the locomotor activity in mice confronted with a free exploratory test, while the drug did not affect the rearing movements or the novelty preference, except at high doses which decreased the time spent by mice in the novel compartment. This latter effect can be interpreted as arising from the so-called "response incompatibility" (Robbins and Iversen 1973) since at these doses the animals exhibited intense and stereotyped locomotion that induced a general behavioral disorganization. Indeed, at high doses, CGS 15943A tended to distribute time randomly between both familiar and novel compartments. By contrast, DPCPX did not significantly modify animals behaviour in this test except at high doses which decreased locomotion and rearing responses, the mice showing ataxia and lack of coordination. When mice were confronted with the light/dark choice procedure which was found to be especially appropriate for revealing anxiolytic and anxiogenic drug-effects (Misslin et al. 1989), CGS 15943A decreased the time spent by mice in the lit box and increased, at the highest dose, the number of transitions, while DPCPX did not significantly alter mice responses. As we chose for this latter test doses of CGS 15943A which did not induce stereotyped locomotion, it can be suggested that here this drug tended to increase the emotional reactivity of mice towards the brightly lighted box of mice as well as their locomotor activity between both boxes. These effects may be due to a non-specific stimulanting action of this drug.

Since CGS 15943A has been presented as an adenosine antagonist with high affinity for both A_1 ($IC_{50}=20$ nM) and A_2 ($IC_{50}=3$ nM) receptors (Ghai et al. 1987; Williams et al. 1987), and DPCPX (Ki = 0.41 nM) as a highly selective A_1-receptor antagonist (Lohse et al. 1987), the present findings suggest that primarily A_2 receptors are involved in the most relevant behavioral drug-effects observed here. This assumption is in agreement with our very recent results which show that highly selective A_2-receptor antagonists were also able to stimulate in a dose-dependent manner locomotor activity in mice (unpublished data). The lack of DPCPX to increase locomotor activity confirm the possible A_2 receptor modulation of locomotor behaviour recently suggested by several authors (Seale et al. 1986; Durean and Morgan 1989; Nikodijevic et al. 1990). Interestingly, Heffner et al. (1989) noted that CV-1808, an adenosine agonist with higher affinity for A_2 as compared with A_1 receptors, and NECA decreased hyperactivity caused by *d*-amphetamine at doses that not cause ataxia while A_1-selective agonists reduced amphetamine's effects only at ataxic doses. On the other hand, Bruns et al. (1988) found that the behavioral effects of CV-1808 were blocked by theophylline, an adenosine antagonist with affinity for both A_1 and A_2 receptors, but not by the selective A_1 antagonist 8-cyclopentyltheophylline. Finally, Spealman and Coffin (1986) observed that the capaci-

ty of adenosine analogs in decreasing schedule-controlled behaviour correlated well with their reported affinities for A_2, but not A_1, recognition sites. In conclusion, the present findings confirm the assumption of Spealman and Coffin (1986) that most of the behavioural effects of adenosine agonists and antagonists are likely mediated by the A_2 receptors.

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