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Adenosine A_1 receptors modulate the anxiolytic-like effect of ethanol in the elevated plus-maze in mice

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Abstract

The anxiolytic property of ethanol is generally accepted to be an important motivational factor for its consumption and the development of alcohol dependence. Recent studies suggest that adenosine receptors mediate important actions of ethanol, such as motor incoordination and hypnotic effects. In addition, several lines of evidence support the involvement of adenosine in anxiety. The aim of the present study was to evaluate the role of adenosine receptors in the anxiolytic-like effect of ethanol in mice. The effects of acute administration of the adenosine receptor antagonists caffeine (nonselective), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, adenosine A_1 receptor antagonist) and 4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo-{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl)phenol (ZM241385, adenosine A_{2A} receptor antagonist), together with the adenosine A_1 receptor agonist 2-chloro-*N*6-cyclopentyladenosine (CCPA), and their interaction with ethanol in the elevated plus-maze test in mice were studied. The highest doses of caffeine (30.0 mg/kg, i.p.) and DPCPX (6.0 mg/kg, i.p.) produced an anxiogenic-like effect, while CCPA administration (0.25 mg/kg, i.p.) showed an anxiolytic-like activity. The prior administration of "non-anxiogenic" doses of caffeine (10.0 mg/kg, i.p.) and DPCPX (3.0 mg/kg, i.p.), but not ZM241385 (1.0 mg/kg, i.p.), significantly reduced the anxiolytic-like effect of ethanol (1.2 g/kg, i.p.). Moreover, anxiolytic-like response was observed by the co-administration of "non-anxiolytic" doses of CCPA (0.125 mg/kg) and ethanol (0.6 g/kg). These results reinforce the involvement of adenosine in anxiety and suggest that the activation of adenosine A_1 receptors, but not adenosine A_{2A} receptors, mediate the anxiolytic-like effect induced by ethanol in mice.

Keywords: Adenosine; Ethanol; Anxiety; Elevated plus-maze; (Mouse)

1. Introduction

Anxiety is generally accepted to be involved in the development of alcohol dependence and relapse. The anxiolytic property of ethanol is one important motivational factor for its consumption, the so-called "tension reduction hypothesis" described initially by Conger (1956), since many alcohol-dependent patients report that they consume alcohol to lessen anxiety (Conger, 1956; Pohorecky, 1981; Newling and Thomson, 1990). The anxiolytic effect of ethanol has been extensively described in different rodent models of anxiety, such as the elevated plus-maze test (Blatt and Takahashi, 1999; Ferreira et al., 2000; LaBuda and Fuchs, 2000, 2002), the light–dark test (Bilkei-Gorzo et al., 1998), the social interaction test (File et al., 1976; Varlinskaya and Spear, 2002) and the free exploratory paradigm (Belzung and Berton, 1997).

An increasing amount of evidence suggests that adenosine receptors mediate important actions of ethanol in both humans and rodents. Adenosine functions as a neuromodulator in the central nervous system (CNS), acting through cell-surface receptors (see Cunha, 2001). Adenosine receptors were recognised on the basis of the ability of caffeine to act as an antagonist at A_1 and A_2 receptors (Snyder et al., 1981). At the moment, four adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) have been cloned and characterised from several mammalian species, including humans and mice, and they all belong to the G-protein-coupled receptor (GPCR) family (see Fredholm et al., 2001).

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Acute exposure to ethanol increases the concentration of extracellular adenosine as a result of ethanol's inhibition of adenosine re-uptake via a facilitative nucleoside transporter (Nagy et al., 1990; Krauss et al., 1993). Furthermore, there is considerable evidence that the co-administration of caffeine can reduce sleep and psychomotor performance impairment associated with moderate-to-high ethanol doses in humans (Franks et al., 1975; Burns and Moskowitz, 1990; Fillmore and Vogel-Sprott, 1995; Liguori and Robinson, 2001; Drake et al., 2003). Moreover, Fillmore (2003) has demonstrated recently that a history of combined alcohol and caffeine administration increased alcohol tolerance compared with an exposure history of either drug alone. In rodents, caffeine and the selective adenosine A_{2A} receptor antagonist 5-amino-7-(β-phenylethyl)-2-(8-furyl)-pyrazolo[4,3-e)-1,2,4-triazolo[1,5-c] pyrimidine (SCH 58261) shortens the duration of the loss of righting reflex induced by ethanol (El Yacoubi et al., 2003). In addition, many studies using selective adenosine receptor agonists and antagonists have demonstrated that adenosine A_1 receptors, localised in brain areas essential for motor control such as the striatum, the cerebellum and the motor cortex, are the primary site where adenosine modulates the incoordination induced by ethanol (Dar et al., 1983; Clark and Dar, 1988; Meng and Dar, 1994, 1995; Barwick and Dar, 1998; Dar, 2001).

Other studies suggest that adenosine receptors can modulate some of the signs of ethanol withdrawal, such as seizures and anxiety. Concas et al. (1994) have described a significant reduction of ethanol withdrawal syndrome in rats after treatment with the selective adenosine A_1 receptor agonist 2-chloro-*N*6-cyclopentyladenosine (CCPA). The absence of the adenosine A_{2A} receptors or their chronic blockade with 4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo-{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl)phenol (ZM241385) results in a decrease of ethanol withdrawal-induced seizures in mice (El Yacoubi et al., 2001).

An increasing number of studies have pointed to a direct involvement of adenosine in anxiety (for review, see Millan, 2003). Mice lacking adenosine A_1 receptors display enhanced anxiety (Johansson et al., 2001; Gimenez-Llort et al., 2002; Lang et al., 2003) and the anxiogenic actions of adenosine receptor antagonists, such as caffeine, in both rodents and humans, have generally been attributed to the blockade of adenosine A_1 receptors (Uhde et al., 1984; Loke et al., 1985; Pellow et al., 1985; File et al., 1988; McCloskey et al., 1990; Jain et al., 1995; Florio et al., 1998). Although no consistent evidence for anxiolytic effects of adenosine A_{2A} receptor stimulation has, to date, been obtained (Jain et al., 1995), some authors have demonstrated that adenosine A_{2A} receptors can exert a facilitatory influence upon γ-aminobutyric acid (GABA) release in the septum and in the hippocampus, actions which may be related to the observation that adenosine A_{2A} receptor-knockout mice score higher than wild-type animals in anxiety tests (Ledent et al., 1997).

The purpose of the present study was to evaluate the role of adenosine receptors in the anxiolytic-like effect induced by ethanol in mice. For this, we investigated the effects of the adenosine receptor antagonists caffeine (nonselective), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, adenosine A_1 receptor antagonist) and ZM241385 (adenosine A_{2A} receptor antagonist), together with the adenosine A_1 receptor agonist CCPA, and their interaction with ethanol in the elevated plus-maze test in mice.

2. Materials and methods

2.1. Animals

A total of 205 male Swiss albino mice weighing 35–45 g from our own colony were used. They were kept in groups of 20 animals per cage and maintained in a room under controlled temperature (23±1 °C). They were subjected to a 12-h light cycle (lights on 7:00 a.m.) with free access to food and water. All tests were carried out between 1100 and 1700 h. All procedures used in the present study complied with the guidelines on animal care of the UFSC Ethics Committee on the Use of Animals which follows the "Principles of laboratory animal care" from NIH publication No. 85-23.

2.2. Drugs

Ethanol (Merck, Brazil) was diluted in 0.9% NaCl (saline) to the concentration of 12% w/v. The adenosine receptor antagonists caffeine (Sigma, USA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and 4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo-{2,3-a}{1,3,5}triazin-5-yl-amino] ethyl)-phenol (ZM241385) (Tocris, USA), together with the adenosine A_1 receptor agonist 2-chloro-*N*6-cyclopentyladenosine (CCPA) (Tocris), were dissolved in saline with 5% dimethylsulfoxide (DMSO). The control solution consisted of saline for ethanol and saline with 5% DMSO (vehicle) for caffeine, DPCPX, ZM241385 and CCPA. All drug doses, selected according to previous literature (Jain et al., 1995; Florio et al., 1998; El Yacoubi et al., 2000), were administered intraperitoneally (i.p.) in a volume of 0.1 ml/10 g of body weight, 30 min before the experiments, except for the ethanol that was administered 15 min before.

2.3. Elevated plus-maze test

The elevated plus-maze was used on the basis of its documented ability to detect both anxiolytic- and anxiogenic-like drug effects in mice (Lister, 1987). Briefly, the apparatus was made of wood covered with impermeable formica and was placed 60 cm above the floor. The four arms were 18 cm long and 6 cm wide. Two opposite arms were surrounded by walls (6 cm high, closed arms), while the other two were devoid of enclosing walls (open arms). The four arms were connected by a central platform (6×6

cm). The experiments were conducted in a sound-attenuated room under low-intensity light (44 lx). Each subject was placed in the centre of the maze facing a closed arm. The animals were observed for a 5-min test period, and anxiolytic-like effects were defined as an increase in the proportion of open-arm entries divided by the total number of arm entries, and the time spent on open arms relative to the total time spent on both arms. Whenever a mouse placed all four paws onto an arm, one entry was recorded. Any decrease in these parameters indicated an anxiogenic-like effect. The total number of closed-arm entries was utilised as a measure of locomotor activity.

2.4. Dose-dependent effects of ethanol in the elevated plus-maze in mice

To evaluate the anxiolytic properties of different ethanol doses in our laboratory, mice were treated intraperitoneally (i.p.) with ethanol (0.6, 1.2 or 2.4 g/kg) or saline (i.p.). After 15 min, the animals were placed in the centre of the elevated plus-maze where their behavioural parameters (described above) were recorded over a period of 5 min.

2.5. Role of adenosine receptors in anxiety and their involvement in the anxiolytic-like effect of ethanol in the elevated plus-maze

Firstly, we investigated the effect of acute administration of the adenosine receptor antagonists caffeine (nonselective, 10.0 or 30.0 mg/kg, i.p.), DPCPX (adenosine A_1 receptor antagonist, 3.0 or 6.0 mg/kg, i.p.), ZM241385 (adenosine A_{2A} receptor antagonist, 1.0 or 3.0 mg/kg, i.p.) or their vehicle (i.p.) in the elevated plus-maze in mice.

A subsequent experiment was performed to evaluate the involvement of adenosine receptors in the anxiolytic-like effect of ethanol in the elevated plus-maze. Thus, we administered selected doses of the adenosine receptor antagonists (based on the previous experiment) caffeine (10.0 mg/kg, i.p.), DPCPX (3.0 mg/kg, i.p.) or ZM241385 (1.0 mg/kg, i.p.) or vehicle (i.p.) 15 min prior to ethanol injection (1.2 g/kg, i.p.). The animals were submitted to elevated plus-maze performance 15 min after ethanol administration.

2.6. Anxiolytic-like responses induced by the combined administration of the adenosine A_1 receptor agonist CCPA and ethanol in the elevated plus-maze

To investigate a possible synergistic interaction between the adenosine A_1 receptor agonist CCPA and ethanol in anxiolytic responses, we first evaluated the effect of a single administration of different CCPA doses (0.125, 0.25, 0.5 or 1.0 mg/kg, i.p.) in the elevated plus-maze. Then, we administered a selected dose of CCPA (0.125 mg/kg, i.p.) 15 min prior to ethanol injection (0.6 g/kg, i.p.), and the animals were tested 15 min later in the elevated plus-maze.

2.7. Statistical analysis

All values are expressed as means ± S.E.M. The statistical comparison of results was carried out using analysis of variance (ANOVA) with treatment as independent variable. Dependent variables were the percentages of entries and time spent on the open arms, and the frequency of enclosed-arm entries. Following significant ANOVAs, multiple post hoc comparisons were performed using the Newman–Keuls test. The accepted level of significance for the tests was $P \leq 0.05$. All tests were performed using the Statistica® software package.

3. Results

3.1. Dose-dependent effects of ethanol in the elevated plus-maze

The results of ethanol administration (0.6, 1.2 or 2.4 g/kg, i.p.) in the behavioural parameters available in the elevated plus-maze are given in Table 1. One-way ANOVA revealed a significant effect for the treatment factor in the percentage of time spent on open arms [$F(3,24) = 25.54$; $P < 0.0001$] and open-arm entries [$F(3,24) = 7.68$; $P = 0.0009$]. However, it also indicated a nonsignificant effect of treatment in the number of closed-arm entries [$F(3,24) = 1.61$; $P = 0.21$] (Table 1).

Subsequent Newman–Keuls tests indicated that ethanol at doses of 1.2 and 2.4 g/kg (i.p.) significantly increased the mouse's exploration of the open arms of the maze, without significant change in the frequency of closed-arm entries, demonstrating an anxiolytic-like effect of these ethanol doses (Table 1).

3.2. Role of adenosine receptors in anxiety and their involvement in the anxiolytic-like effect of ethanol in the elevated plus-maze

The effects of the administration of the adenosine receptor antagonists caffeine (10.0 or 30.0 mg/kg, i.p.), DPCPX (3.0 or 6.0 mg/kg, i.p.) or ZM241385 (1.0 or 3.0 mg/kg, i.p.) in the elevated plus-maze can be seen in Fig. 1.

Table 1
Effect of treatment with ethanol in the elevated plus-maze in mice

Treatment	% Open arm time	% Open-arm entries	Enclosed-arm entries
Saline	24.9±1.9	28.5±3.8	8.1±1.4
Ethanol (0.6 g/kg)	23.2±1.6	29.7±3.0	8.7±0.6
Ethanol (1.2 g/kg)	47.5±2.9[a]	44.3±2.6[a]	11.0±1.1
Ethanol (2.4 g/kg)	50.5±4.2[a]	43.7±2.8[a]	11.8±2.1

Ethanol (0.6, 1.2 or 2.4 g/kg) or saline (control) were administered by intraperitoneal route, and 15 min later, the animals were tested in the elevated plus-maze. Each value represents the mean ± S.E.M. of eight animals per group.

[a] $P \leq 0.05$ compared to the saline-treated group (Newman–Keuls test).

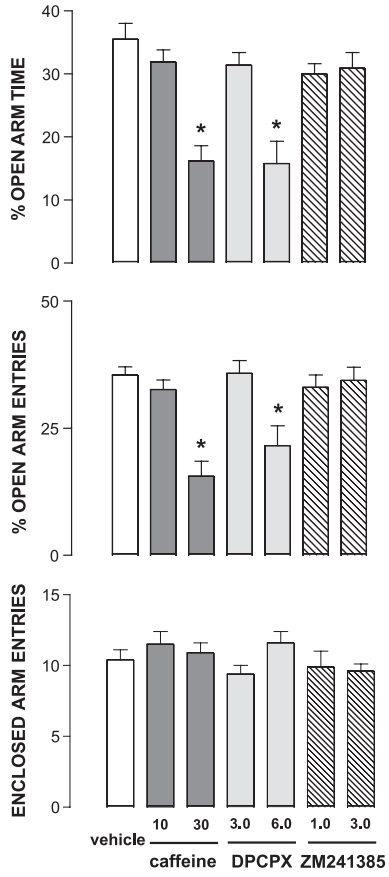

Fig. 1. Effect of treatment with adenosine receptor antagonists in the elevated plus-maze in mice. Caffeine (nonselective, 10.0 or 30.0 mg/kg), DPCPX (adenosine A_1 receptor antagonist, 3.0 or 6.0 mg/kg), ZM241385 (adenosine A_{2A} receptor antagonist, 1.0 or 3.0 mg/kg) or vehicle were injected by intraperitoneal route, and 30 min later, the animals were tested in the elevated plus-maze. Each bar represents the mean ± S.E.M. of eight animals per group. $^{*}P \leq 0.05$ compared to the vehicle-treated group (Newman–Keuls test).

One-way ANOVA revealed a significant effect for the treatment factor in the percentage of time spent on open arms [$F(6,49)=10.58$; $P<0.0001$] and open-arm entries [$F(6,49)=8.64$; $P<0.0001$], without significance for the number of closed-arm entries [$F(6,49)=1.40$; $P=0.23$]. Further comparisons showed that the highest doses of caffeine (30.0 mg/kg, i.p.) and DPCPX (6.0 mg/kg, i.p.) used in the tests significantly reduced the percentages of entries and time spent on open arms (Newman–Keuls test, $P \leq 0.05$), indicating an anxiogenic-like effect (Fig. 1).

Fig. 2 illustrates the effects of the administration of "non-anxiogenic" doses of the adenosine receptor antagonists caffeine (10.0 mg/kg, i.p.), DPCPX (3.0 mg/kg, i.p.) or ZM241385 (1.0 mg/kg, i.p.) on the anxiolytic-like effect induced by ethanol (1.2 g/kg, i.p.). One-way ANOVA revealed a significant effect for the treatment factor in the percentage of time spent on open arms [$F(5,39)=13.39$; $P<0.0001$] and number of open-arm entries [$F(5,39)=10.36$; $P<0.0001$], without significance for the number of closed-arm entries [$F(5,39)=1.58$; $P=0.19$].

Post hoc comparisons demonstrated that the adenosine receptor antagonists caffeine (10.0 mg/kg, i.p.), DPCPX (3.0 mg/kg, i.p.) and ZM241385 (1.0 mg/kg, i.p.) did not alter the behavioural parameters in the elevated plus-maze when administered alone. However, the prior administration of these same doses of caffeine or DPCPX, but not ZM241385, were able to block the anxiolytic-like effect of ethanol (1.2 g/kg, i.p.) (Fig. 2).

3.3. Anxiolytic-like responses induced by the combined administration of the adenosine A_1 receptor agonist CCPA and ethanol in the elevated plus-maze

The effects of adenosine A_1 receptor agonist CCPA injection (0.125, 0.25, 0.5 or 1.0 mg/kg, i.p.) in the elevated

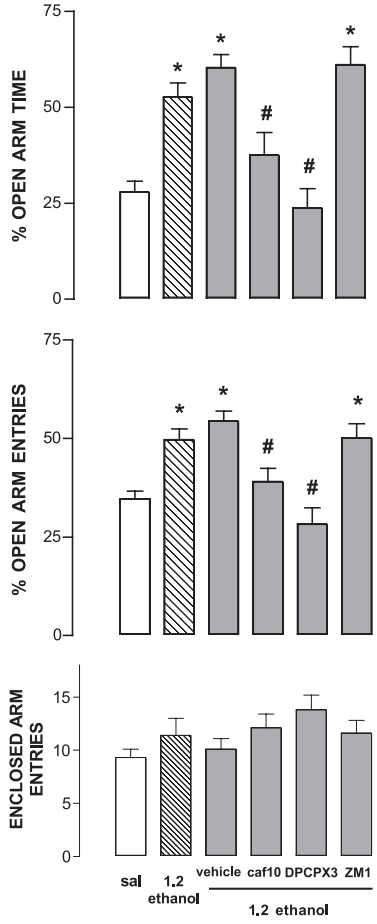

Fig. 2. Effect of treatment with adenosine receptor antagonists in the anxiolytic-like effect induced by ethanol in mice. "Non-anxiogenic" doses of the adenosine receptor antagonists caffeine (10.0 mg/kg, i.p.), DPCPX (3.0 mg/kg, i.p.), ZM241385 (1.0 mg/kg, i.p.) or vehicle (i.p.) were injected 15 min before ethanol (1.2 g/kg, i.p.), and 15 min later, the animals were tested in the elevated plus-maze. Each bar represents the mean ± S.E.M. of seven to eight animals per group. $^{*}P \leq 0.05$ compared to the saline-treated group. $^{\#}P \leq 0.05$ compared to the group that received only ethanol (1.2 g/kg, i.p.) (Newman–Keuls test).

plus-maze are illustrated in Fig. 3. CCPA administration promoted a significant effect (ANOVA) on the percentage of time spent on open arms [$F(4,34)=6.05$; $P=0.0009$], the percentage of open-arm entries [$F(4,34)=6.31$; $P=0.0006$] and the number of closed-arm entries [$F(4,34)=12.12$; $P<0.0001$]. Subsequent Newman–Keuls tests indicated that CCPA, at a dose of 0.25 mg/kg (i.p.), produced an increase in the exploration of the open arms of the elevated plus-maze, with no change in the frequency of closed-arm entries, indicating an anxiolytic-like effect of this CCPA dose. Also, further comparisons between groups revealed that the high doses of CCPA (0.5 and 1.0 mg/kg, i.p.) significantly decreased the locomotor activity of mice compared to the control group, as shown by the reduced total number of closed-arm entries (Fig. 3).

As can be seen from Fig. 4, the administration of a "non-anxiolytic" dose of CCPA (0.125 mg/kg, i.p.) 15 min prior to the injection of a "non-anxiolytic" dose of ethanol (0.6 g/kg, i.p.) promoted a significant increase (ANOVA) in the percentage of time spent on open arms [$F(3,23)=17.58$; $P<0.0001$] and open-arm entries [$F(3,23)=34.28$; $P<0.0001$], without significance for the number of closed-arm entries [$F(3,23)=1.51$; $P=0.24$], indicating an enhancement of anxiolytic-like response following the combined administration of the adenosine A_1 receptor agonist CCPA and ethanol in the elevated plus-maze in mice (Fig. 4).

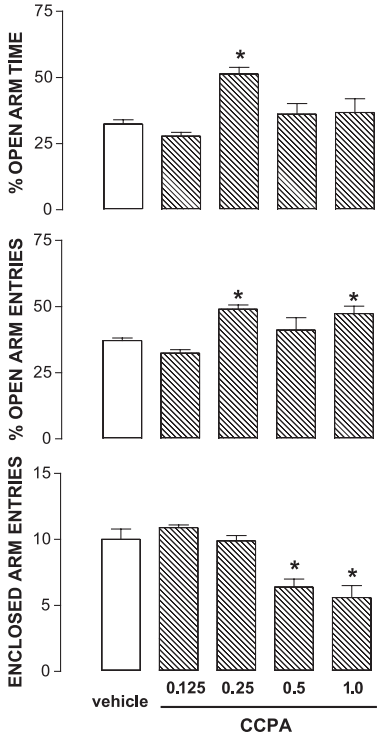

Fig. 3. Effect of treatment with the adenosine A_1 receptor agonist CCPA in the elevated plus-maze in mice. CCPA (0.125, 0.25, 0.5 or 1.0 mg/kg.) or vehicle were administered intraperitoneally, and 30 min later, the animals were tested in the elevated plus-maze. Each bar represents the mean ± S.E.M. of eight animals per group. *$P \leq 0.05$ compared to the vehicle-treated group (Newman–Keuls test).

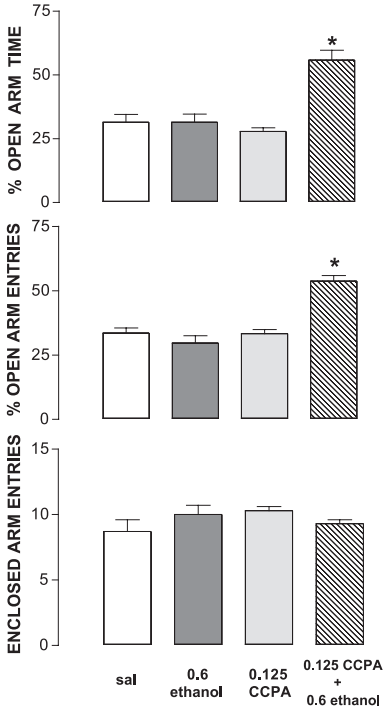

Fig. 4. Interaction between the adenosine A_1 receptor agonist CCPA and ethanol in anxiolytic-like responses in mice. CCPA (0.125 mg/kg, i.p.) was administered 15 min before ethanol (0.6 g/kg, i.p.), and the animals were tested 15 min later in the elevated plus-maze. Each bar represents the mean ± S.E.M. of eight to nine animals per group. *$P \leq 0.05$ compared to the saline-treated group (Newman–Keuls test).

4. Discussion

The present findings reinforce the idea of the involvement of adenosine in anxiety since they confirm the existence of anxiogenic-like responses induced by caffeine (30.0 mg/kg, i.p.) and the selective adenosine A_1 receptor antagonist DPCPX (6.0 mg/kg, i.p) in the elevated plus-maze in mice, while the adenosine A_1 receptor agonist CCPA (0.25 mg/kg, i.p.) shows an anxiolytic-like profile in this paradigm. More importantly, our results demonstrate for the first time that the anxiolytic-like effect induced by ethanol (1.2 g/kg, i.p.) in mice is modulated by adenosine A_1 receptors (but not by A_{2A} receptors), since this response is blocked by the previous administration of "non-anxiogenic" doses of caffeine (10.0 mg/kg, i.p.) and DPCPX (3.0 mg/kg, i.p.), but not by ZM241385 (1.0 mg/kg, i.p.). Furthermore, the present data indicate an enhanced effect following the administration of "non-anxiolytic" doses of CCPA (0.125 mg/kg, i.p.) and ethanol (0.6 g/kg, i.p) in the development of anxiolytic-like responses in mice.

Consistent with previous studies that have demonstrated the anxiogenic properties of high doses of caffeine in different animal models of anxiety, such as the social

interaction test (Baldwin et al., 1989; Bhattacharya et al., 1997), the elevated plus-maze (Pellow et al., 1985; Baldwin et al., 1989; El Yacoubi et al., 2000) and the light–dark test (Imaizumi et al., 1994; El Yacoubi et al., 2000), we found that the highest dose of caffeine tested (30.0 mg/kg, i.p.) decreased the frequency of entries and the time spent in the open arms by mice in the elevated plus-maze. However, these anxiogenic-like actions could not be demonstrated for the low dose of caffeine tested (10.0 mg/kg, i.p.).

In the current study, we present evidence that DPCPX, a selective adenosine A_1 receptor antagonist, exerts an anxiogenic-like effect in mice at the highest dose tested (6.0 mg/kg, i.p.) with no significant effect on the elevated plus-maze at the lowest dose tested (3.0 mg/kg, i.p.). This result contrasts with previous studies that have failed to show anxiety-related behaviours in the elevated plus-maze in mice after the treatment with DPCPX (Jain et al., 1995; El Yacoubi et al., 2000). However, in these studies the low range of DPCPX doses (0.05–5.0 mg/kg) used by the authors may explain, in part, the discrepancy with the present results, since we also did not detect any anxiolytic-like activity of DPCPX at the low dose tested (3.0 mg/kg, i.p.). Furthermore, in accordance with a previous study of Florio et al. (1998), the present results indicate an anxiolytic-like effect of CCPA (0.25 mg/kg, i.p.), a highly selective adenosine A_1 receptor agonist. On the other hand, as previously demonstrated by El Yacoubi et al. (2000), the administration of the selective adenosine A_{2A} receptor antagonist ZM241385 (1.0 or 3.0 mg/kg, i.p.) did not affect any of the parameters that were recorded in the elevated plus-maze, indicating a lack of ZM241385 effect in this model of anxiety, at least at the dosage range tested. Taken together, these findings are in agreement with earlier studies (Jain et al., 1995; Florio et al., 1998; Johansson et al., 2001; Gimenez-Llort et al., 2002; Lang et al., 2003) that have shown the involvement of adenosine receptors (mainly A_1 receptors) on the control of anxiety-related responses in mice. However, it must be conceded that additional study with other selective antagonists for adenosine A_{2A} receptors would be necessary in order to discard completely the involvement of this receptor subtype in anxiety.

In addition, the present study demonstrates that the acute administration of "non-anxiogenic" doses of the adenosine receptor antagonists caffeine (10.0 mg/kg, i.p.) and DPCPX (3.0 mg/kg, i.p.), but not ZM241385 (1.0 mg/kg, i.p.), blocks the anxiolytic-like actions of ethanol (1.2 g/kg, i.p.) in the elevated plus-maze. Although a possible pharmacokinetic interaction between the adenosine antagonists and ethanol cannot be ruled out, since plasma ethanol concentrations were not measured, we believe that the present results reflect a functional interaction. Previous study has demonstrated that the deficiency of adenosine receptors in knockout mice does not cause any significant alteration in blood ethanol levels (El Yacoubi et al., 2001). Moreover, corroborating our functional interaction hypothesis between adenosine A_1 receptors and ethanol in anxiety-related actions, the present results indicate a synergistic response induced by "non-anxiolytic" doses of the adenosine A_1 receptor agonist CCPA (0.125 mg/kg, i.p.) and ethanol (0.6 g/kg, i.p.) that produced a marked increase in the frequency of entries and the time spent in the open arms of the maze by mice.

Consistent with the present data, an increasing amount of evidence from several laboratories has pointed to a direct role for the neuromodulator adenosine in mediating some of the cellular and behavioural responses to ethanol in both humans and rodents. Acute exposure to ethanol increases the concentration of extracellular adenosine (Nagy et al., 1990; Krauss et al., 1993). Furthermore, many studies have confirmed the popular belief that caffeine can antagonise the intoxicating effects, such as sleep and motor incoordination, induced by moderate to high doses of ethanol in humans (Franks et al., 1975; Burns and Moskowitz, 1990; Fillmore and Vogel-Sprott, 1995; Liguori and Robinson, 2001; Drake et al., 2003). Moreover, the combined administration of caffeine and alcohol can increase the development of alcohol tolerance (Fillmore, 2003). In rodents, it is known that caffeine and selective adenosine receptor antagonists reduce the duration of the ethanol-induced loss of the righting reflex (El Yacoubi et al., 2003) and block the motor incoordination promoted by ethanol (Dar et al., 1983; Clark and Dar, 1988; Dar, 2001; Meng and Dar, 1994, 1995; Barwick and Dar, 1998). In addition, other studies suggest a reduction of the ethanol withdrawal syndrome in rats after treatment with the adenosine A_1 receptor agonist CCPA (Concas et al., 1994) and a decrease of ethanol withdrawal-induced seizures in mice either lacking the adenosine A_{2A} receptor altogether or after its chronic blockade with ZM241385 (El Yacoubi et al., 2001).

The present finding that adenosine A_1 receptors modulate anxiolytic-like actions of ethanol suggests a speculative hypothesis that selective adenosine A_1 receptor agonists might represent an important therapeutic tool to ameliorate the anxiogenic effects of ethanol withdrawal, reducing the recidivism in ethanol addicts. To our knowledge, only one study so far has tested the potential of adenosine A_1 receptor agonists in the treatment of ethanol withdrawal-induced anxiety. Gatch et al. (1999) investigated the effect of the adenosine A_1 receptor agonist *R*(−)-*N*6-(2-phenyl-isopropyl)adenosine (R-PIA) and the 8-cyclopentyl-1,3-dimethylxanthine (CPT), an adenosine A_1 receptor antagonist, on the ethanol-induced withdrawal in rats after a "chronic" exposure to ethanol for 7 days. Surprisingly, the authors demonstrated opposite effects to those expected, with a decrease of time spent in the open arms in the animals treated with R-PIA, suggesting an anxiogenic-like effect, while the A_1 receptor antagonist CPT showed an anxiolytic effect during ethanol withdrawal. The explanation proposed by the authors for the lack of A_1 agonist R-PIA anxiolytic effect is that the number of adenosine receptors is markedly decreased during ethanol withdrawal (Dar et al., 1983) and that there may not be sufficient receptors to produce an

effect even when fully occupied. However, they did not discard the importance of additional research to verify the effects of adenosine compounds on ethanol withdrawal.

In conclusion, the present results confirm and extend the involvement of adenosine in the control of anxiety, showing that the anxiolytic-like effect induced by ethanol in mice is modulated by adenosine A_1 receptors. Additional research is needed to clarify whether the adenosine A_1 receptors are involved in the anxiolytic actions of ethanol and to investigate whether there exists any interaction between the adenosinergic and other neurotransmission systems in the present effects of adenosine antagonists. Finally, a better evaluation of the potential of adenosine A_1 receptor agonists to reduce the anxiogenic effects during ethanol withdrawal is also indicated.

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