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SCH 58261 and ZM 241385 differentially prevent the motor effects of CGS 21680 in mice: evidence for a functional 'atypical' adenosine A_{2A} receptor

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Abstract

The acute motor effects elicited by drugs acting upon adenosine A_{2A} receptors, namely the highly selective agonist CGS 21680 or the antagonists SCH 58261 and ZM 241385, were investigated in mice. CGS 21680 dose-dependently (0.1–2.5 mg/kg i.p.) decreased horizontal and vertical motor activities. The depressant effect of CGS 21680 (0.5 mg/kg i.p.) was maintained in mice pretreated by the adenosine receptor antagonist 8-(*p*-sulfophenyl)-theophylline (10–30 mg/kg i.p.), which poorly penetrates the blood–brain barrier, but was completely lost in adenosine A_{2A} receptor knockout mice. Thus, the adenosine A_{2A} receptor is critically involved in motor activity. SCH 58261 (1–10 mg/kg i.p.) increased locomotion and rearing with a quick onset, but for a shorter period in mice habituated to the environment than in mice unfamiliar to it. ZM 241385 (7.5–60 mg/kg i.p.) stimulated horizontal and vertical activities with a slow onset at the two highest tested doses, similarly in naive and in habituated mice. The increase in locomotion elicited by ZM 241385 (15–30 mg/kg i.p. and 10–20 nM i.c.v.) was retained in mice treated by CGS 21680 (0.5 mg/kg i.p.) but that elicited by SCH 58261 (1–3–10 mg/kg i.p. and 10–20 nM i.c.v.) partially subsided. In conclusion, both 'striatal-like'/'SCH 58261-sensitive' adenosine A_{2A} receptors and 'ZM 241385-sensitive'/'atypical' CGS 21680 binding sites may mediate CGS 21680-induced motor effects. Moreover, our results suggest that 'atypical' CGS 21680 binding sites could be adenosine A_{2A} receptors with a peculiar pharmacological profile.

Keywords: Adenosine A_{2A} receptor; SCH 58261; ZM 241385; CGS 21680; Motor activity; Knockout, mouse

1. Introduction

Adenosine represents an endogenous inhibitory modulator that is widely distributed in the mammalian central nervous system (CNS) at concentrations ranging from 30 to 300 nM (Fredholm, 1995). Specificity in its central actions appears to depend on the discrete distributions of adenosine receptor subtypes in the brain. To date, the receptors for adenosine have been pharmacologically and structurally classified into A_1, A_{2A}, A_{2B}, and A_3 (reviewed by Fredholm et al., 1994). Pharmacological actions at adenosine A_{2A} receptors offers an attractive target for treatment of several human CNS diseases (Ongini and Fredholm, 1996). For example, adenosine A_{2A} receptor antagonists may have potential as antiparkinsonian drugs (Fenu et al., 1997; Kanda et al., 1998), a hypothesis supported by in vivo pharmacological evidence for a role of adenosine in controlling motor functions (Snyder et al., 1981; Durcan and Morgan, 1989a; Nikodijevic et al., 1991; Janusz and Berman, 1993; Barraco et al., 1994; Hauber and Münkle, 1997; Ledent et al., 1997). Adenosine A_{2A} receptor antagonists also reduce cell death in animal models of cerebral ischaemia (Monopoli et al., 1998). For CNS drugs, the expression of a centrally mediated behaviour after peripheral administration can provide a valuable first indication of clinical potential. Motor activity is a centrally mediated behaviour that can be modified by pharmacological tools and continues to be a simple and widely used behavioural assay in investigating the effects of adenosine

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receptor ligands. The most typical feature of behaviour observed in laboratory animals following adenosine receptor activation is the suppression of exploratory motor activity (Snyder et al., 1981; Katims et al., 1983; Durcan and Morgan, 1989a). Conversely, the prototypic effect of adenosine receptor antagonism is an increase in motor activity (Griebel et al., 1991a,b; Brockwell and Beninger, 1996). The ability to suppress motor activity appears to be shared by three adenosine receptor subtypes A_1, A_{2A}, and A_3 (Nikodijevic et al., 1991; Jacobson et al., 1993a) and to be centrally mediated, since adenosine receptor agonists administered into the brain are active (Barraco and Bryant, 1987; Barraco et al., 1994), whereas peripherally selective adenosine receptor antagonists are ineffective in blocking the suppression of motor activity (Durcan and Morgan, 1989b; Marston et al., 1998). The classical adenosine receptor antagonist caffeine causes biphasic and dose-dependent effects on motor behaviour: low doses increase and high doses decrease motor activity (Waldeck, 1975; Svenningsson et al., 1995; El Yacoubi et al., 2000b). It has been shown that the motor-increasing effects of caffeine result from a competitive interaction at adenosine A_{2A} receptors (Fredholm, 1999), because the stimulant effects of caffeine are turned into a behavioural depression in adenosine A_{2A} receptor knockout mice (Ledent et al., 1997; El Yacoubi et al., 2000b). In addition, the xanthine derivative 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), which shows about 500-fold selectivity for adenosine A_1 receptor vs. adenosine A_{2A} receptor, has been found to produce only decreases in motor activity at high doses (Griebel et al., 1991a; Svenningsson et al., 1997; El Yacoubi et al., 2000b). However, its close structural analogue 8-cyclopentyl-1,3-dimethylxanthine (CPT), which is a less selective adenosine A_1 receptor antagonist, was shown to increase motor activity in rats (Popoli et al., 1998). Taken together, the analysis of the pharmacological profile of both adenosine receptor agonists and antagonists to alter motor activity has led to the notion that the suppression of motor activity is primarily mediated by an activation of adenosine A_{2A} receptors since adenosine A_2 receptor agonists are generally more potent than adenosine A_1 receptor agonists in reducing locomotion (Durcan and Morgan, 1989a; Barraco et al., 1994). Central adenosine A_{2A} receptors located in striatum and nucleus accumbens might be responsible for the effects upon rearing and locomotion, respectively (Barraco and Bryant, 1987; Barraco et al., 1994). We speculated that two adenosine A_{2A} receptor antagonists SCH 58261 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4,-triazolo-[1,5-c]-pyrimidine (Zocchi et al., 1996) and ZM 241385 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]-ethyl)phenol (Poucher et al., 1995; Keddie et al., 1996) might also be interesting tools to provide conclusive evidence for a role of adenosine A_{2A} receptor in regulating motor activity. These two drugs have different degrees of selectivity over adenosine A_{2B} receptors, since only SCH 58261 shows negligible interaction with adenosine A_{2B} receptors in vitro (Ongini et al., 1999). Another difference that has been observed using in vitro assays about the pharmacological profiles of these two antagonists is the ability of SCH 58261 (Lindström et al., 1996) but not of ZM 241385 (Cunha et al., 1997) to discriminate between two different binding sites of the selective adenosine A_{2A} receptor agonist CGS 21680 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine (Jarvis et al., 1989). We were, therefore, interested in comparing the motor responses induced by SCH 58261 or ZM 241385 in absence or presence of CGS 21680, which may help to confirm these different profiles using an in vivo approach. To this end, the motor effects of CGS 21680 were studied in our experimental conditions. Except for caffeine and CGS 21680, the effects of drugs acting on adenosine A_{2A} receptors upon the vertical component of motor activity (rearing) are poorly documented (Griebel et al., 1991b; Rimondini et al., 1997; Svenningsson et al., 1997) and were also studied. Adenosine is involved in the regulation of sleep-wake phenomena (Porkka-Heiskanen, 1999). As we have recently shown that selective adenosine A_{2A} receptor antagonists SCH 58261 and ZM 241385 do not share the anxiogenic-like effects of caffeine (El Yacoubi et al., 2000a), it was also of interest to compare their motor effects in two experimental conditions where the degree of arousal of the animals and the aversive content of their environment was different.

2. Materials and methods

2.1. Animals

Male Swiss albino CD1 mice (Charles River, Saint Aubin lès Elbeuf, France) or adenosine 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 one week of habituation in our own facilities. Mice were housed in groups of 15–20 in Makrolon cages ($38 \times 24 \times 18$ cm) with free access to water and food (U.A.R., France) and kept in a ventilated room at a temperature of 21 ± 1°C, under a 12 h light/12 h dark cycle (light on between 0700 and 1900 h). Experiments were carried out between 0900 and 1900 h. The animals were isolated in small individual cages ($27 \times 13 \times 13$ cm) for 30 min prior testing.

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

2.2. Behavioural analysis

2.2.1. Motor activity assessment in CD1 mice

Motor activity of CD1 mice was measured with a Digiscan Animal Activity Monitor system (Omnitech Elec-

tronics, Columbus, OH, USA), which monitored the horizontal (locomotion) and vertical (rearing) movements of the animals (Michael-Titus et al., 1987). The Digiscan analyser was interfaced with an 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 and vertical motor activities were expressed as number of beams crossed over the two, three or four 15-min sessions of testing. Usually, mice were introduced into the actometers without habituation to the test arena (nonhabituated mice). In two experiments, the mice were removed from their individual cages and placed individually in the motility cages for a 30-min or 1-h habituation session. This habituation procedure was applied so that the subject's activity could be recorded during the subsequent experimental session without the interference of spontaneous exploratory behaviour. Habituated mice were then injected with adenosine A_{2A} receptor antagonists or vehicle, immediately returned to the motility cages and motor activities were recorded.

2.2.2. Motor activity assessment in wild-type and adenosine A_{2A} receptor knockout mice

The first experimental device was a square (40×40), open-field painted black, surrounded by walls (30 cm high) covered with a fine wire-netting top. The testing session lasted 30 min and was preceded by a 5-min period of habituation (Simon et al., 1994). The second apparatus was an elevated plus-maze, i.e. a wooden Greek cross, painted black, and placed 60 cm above the floor (Ledent et al., 1997). Only the total distance travelled on the maze over a 5-min session was analysed in the present experiment. The measures were automated using an image analysis system (Videotrack 512 system, Viewpoint, Lyon, France).

2.3. Intracerebroventricular injections

Mice were manually immobilized and i.c.v. injections (10 μl in about 3 s) were made free-hand into the left ventricle, as described by Haley and Mc Cormick (1957). A microsyringe (Hamilton, 50 μl) with a needle 0.4 mm in diameter and protruding 3.5 mm from a guard (to fix penetration into the brain) was used.

2.4. Drugs

CGS 21680 (2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine HCl) and 8-(*p*-sulfophenyl)-theophylline (8-SPT) were purchased from Sigma. DPCPX was purchased from RBI (Natick, MA, USA). ZM 241385 and SCH 58261 were generous gifts from Dr. S. Poucher (Zeneca Pharmaceuticals, Macclesfield, U.K.) and Dr. E. Ongini (Schering-Plough Research Institute, Milan, Italy), respectively. Except for 8-SPT that was dissolved in NaCl 0.9%, all other compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma) and then diluted in Cremophor EL (Sigma) and NaCl 0.9% (final concentration: 15% DMSO and 15% Cremophor EL). The drug solutions were prepared fresh daily and injected i.p. in a volume of 10 ml/ kg or i.c.v. in a volume of 10 μl.

2.5. Statistics

Results are expressed as means ± S.E.M.. Statistically, these data were tested first within a group of experiments (i.e. habituated or nonhabituated mice) by means of two-way analysis of variance (ANOVA) for repeated measures using the time of testing (three or four levels) and the doses as between factors and subjects as within factors. 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 Newman–Keuls tests for multiple comparisons to compare treated groups vs. the respective control group. In a second level of data analysis, we asked whether the motor effects of adenosine A_{2A} receptor antagonists obtained after habituation to the Digiscan apparatus differed from those observed in nonhabituated mice. Therefore, we compared the two experiments

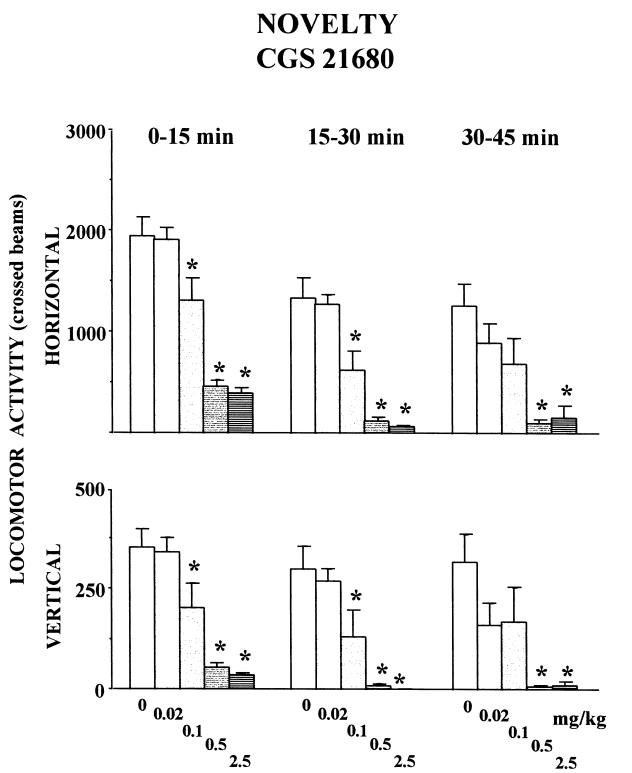

Fig. 1. Effect of the adenosine A_{2A} receptor agonist CGS 21680 on motor activity in nonhabituated mice. Mice were injected with vehicle (open bars) or increasing doses of CGS 21680 (0.02–0.1–0.5–2.5 mg/kg i.p. hatched bars from grey to black) and were introduced into the actometers. The horizontal (upper panel) and vertical (lower panel) components of motor activity were measured for 45 min. Means ± S.E.M. of data from 11–12 mice/group. $^{*}P < 0.05$ (by Newman–Keuls post hoc test following a one-way ANOVA).

using an additional ANOVA for repeated measures using 'habituation' (two levels) and drugs as between factors, and animals as within factor. A Kruskal–Wallis one-way ANOVA on ranks combined with a Dunn's multiple comparison test was used to analyse non parametric data. In the final experiment, we compared the effects of three drugs using a two-way ANOVA. Significance levels were set at $P < 0.05$.

3. Results

3.1. Stimulation of adenosine A_{2A} receptors by CGS 21680

Fig. 1 shows the time course of the motor activity response to acute administration of the selective adenosine A_{2A} receptor agonist CGS 21680 (0.02–0.1–0.5–2.5 mg/kg i.p.) in mice nonhabituated to the open field. A two-way ANOVA with repeated measures over time revealed that the effects upon horizontal activity (dose × time: $F(8,167) = 3.73$, $P < 0.001$) and vertical activity (dose × time: $F(8,167) = 2.19$, $P < 0.05$) were time-dependent. The results of separate ANOVAs followed by multiple comparisons using Newman–Keuls post hoc test indicated that both components of motor activity were significantly reduced in comparison to control mice at 0.5 and 2.5 mg/kg. At the intermediate 0.1 mg/kg dose, however, activity was significantly decreased only for 30 min after injection.

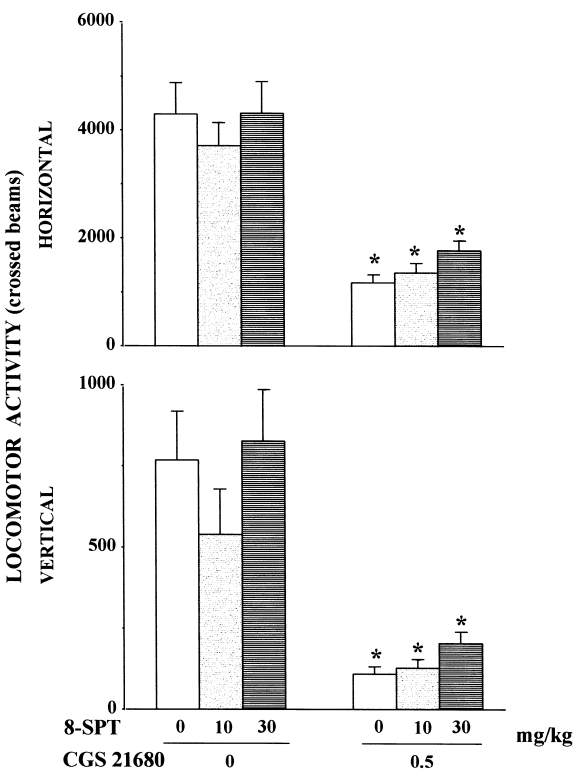

Fig. 2. Effect of the peripherally acting adenosine receptor antagonist 8 phenyl sulfo-theophylline (8-PST) on inhibition of motor activity induced by CGS 21680 in nonhabituated mice. Mice were injected with vehicle (open bars) or 8-SPT, (10–30 mg/kg i.p. hatched bars grey and black). Fifteen minutes later, they were injected with vehicle or CGS 21680 (0.5 mg/kg i.p.) and were introduced into the actometers. The horizontal (upper panel) and vertical (lower panel) components of motor activity were measured for 60 min. Means ± S.E.M. of data from 9–10 mice/group. * $P < 0.001$ as compared with respective control (by Newman–Keuls post hoc test following a two-way ANOVA).

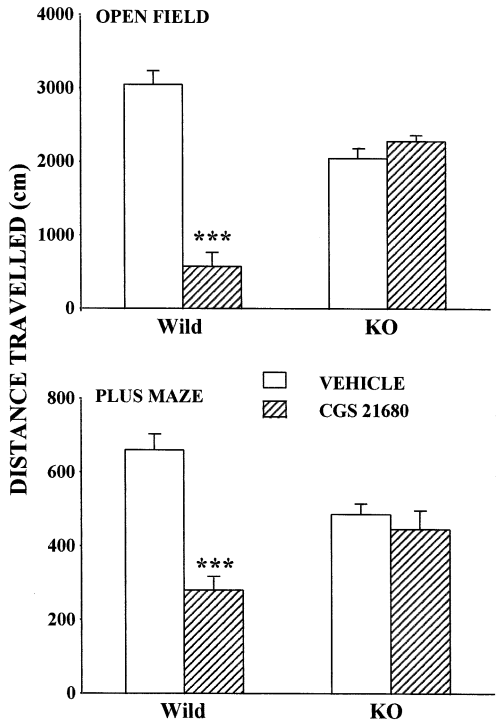

Fig. 3. Effects of CGS 21680 on motor activity in wild-type and adenosine A_{2A} receptor knockout mice assessed in open-field or in elevated plus maze. Mice were injected with vehicle (open bars) or CGS 21680 (0.5 mg/kg i.p. hatched bars). Exploratory behaviour was recorded 30 min after administration of drugs for a 10-min session after a 5-min period of habituation, in a square open-field (upper panel) and for a 5-min session in an elevated plus maze (lower panel). Means ± S.E.M. of data from 20 controls and 8 CGS 21680-injected mice in open-field test and 33 controls and 16 CGS 21680-injected mice in plus-maze test. * $P < 0.001$ as compared with respective control (by Student's *t*-test).

The effect of 8-SPT (10-30 mg/kg i.p.) on motor response elicited by CGS 21680 (0.5 mg/kg i.p.) in nonhabituated mice is shown in Fig. 2. There was no CGS 21680 × 8-SPT interaction in this experiment for any of the two measured parameters: horizontal activity, [$F(2,218) = 0.58$, $P > 0.05$]; vertical activity, [$F(2,218) = 1.43$, $P > 0.05$]. The analysis of the horizontal and vertical components of motor activity in these mice showed a main effect of CGS 21680 upon horizontal activity [$F(1,218) = 76.62$, $P < 0.001$] and rearing [$F(1,218) = 76.89$, $P < 0.001$] whether the peripheral adenosine receptor antagonist 8-SPT was present or not (post hoc tests). At the two tested doses, 8-SPT itself was devoid of effect upon locomotion [$F(2,218) = 0.4$, $P > 0.05$], and vertical activity [$F(2,218) = 2.61$, $P > 0.05$].

In previous behavioural studies, adenosine A_{2A} receptor knockout mice appeared to be less active than wild-type control mice in the automated open-field apparatus used in the present experiments. Moreover, it was shown that the motor depressant effects induced by CGS 21680 (0.5 mg/kg i.p.) in wild-type mice were absent in adenosine A_{2A} receptor knockout mice (Ledent et al., 1997). The selective adenosine A_{2A} receptor agonist produced similar effects in two paradigms in which motor activity was assessed using a different video-tracking system, as shown in Fig. 3. As compared with vehicle-injected animals, the total distance travelled in a weakly illuminated square open-field was significantly decreased [$P < 0.001$] in wild-type mice receiving CGS 21680 (0.5 mg/kg i.p.) 30 min before the test (Fig. 3, upper panel) but remained unchanged [$P > 0.05$] in knockout mice. The selective adenosine A_{2A} receptor agonist also produced a powerful depressant effect [$P < 0.001$] upon locomotion in wild-type mice placed onto an elevated plus-maze in an experiment designed to assess anxiolytic properties of drugs, whereas it remained inactive [$P > 0.05$] in adenosine A_{2A} receptor knockout mice (Fig. 3, lower panel).

3.2. Blockade of adenosine A_{2A} receptors by SCH 58261

Fig. 4 shows that the selective adenosine A_{2A} receptor antagonist SCH 58261 (1–3–10 mg/kg i.p.) time-dependently increased both components of motor activity [horizontal activity (dose × time: $F(9,263) = 4.49$, $P < 0.001$); vertical activity (dose × time: $F(9,263) = 2.69$, $P < 0.01$)] in nonhabituated mice. In the control group of mice, horizontal activity gradually decreased during the first 30 min and stabilised thereafter. SCH 58261 dose-dependently increased horizontal activity up to 10 mg/kg. Es-

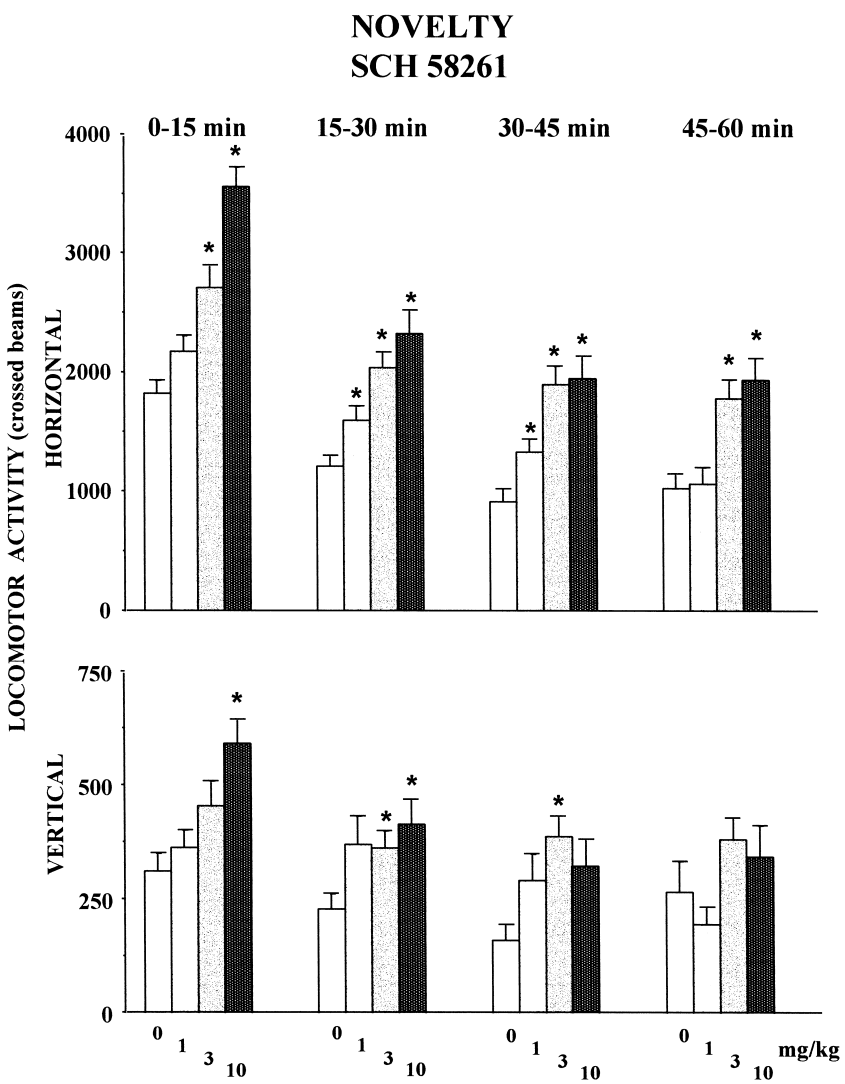

Fig. 4. Effects of the adenosine A_{2A} receptor antagonist SCH 58261 on motor activity in nonhabituated mice. Mice were injected with vehicle (open bars) or increasing doses of SCH 58261 (1–3–10 mg/kg i.p. hatched bars from grey to black) and were introduced into the actometers. The horizontal (upper panel) and vertical (lower panel) components of motor activity were measured for 60 min. Means ± S.E.M. of data from 21 controls and 15 mice in treated groups. $^{*}P < 0.05$ (by Newman–Keuls post hoc test following a one-way ANOVA).

sentially at this highest tested dose, vertical activity (rearing) was also significantly enhanced up to 30 min post-injection. Another experiment was performed to study the influence of novelty stress upon the SCH 58261-induced motor response. In mice habituated to their environment (Fig. 5), SCH 58261 also time-dependently (dose × time: $F(9,207) = 5.07$, $P < 0.001$) increased horizontal activity, but only during the first 45 min of the experiment. Vertical activity was time-dependently (dose × time: $F(9,207) = 3.54$, $P < 0.001$) increased at the 3 mg/kg dose of SCH 58261 only for the first 15 min of the experiment.

3.3. Blockade of adenosine A_{2A} receptors by ZM 241385

The acute administration of the adenosine A_{2A} receptor antagonist ZM 241385 (7.5–15–30–60 mg/kg i.p.) time-dependently enhanced horizontal (dose × time: $F(12,559) = 4.53$, $P < 0.001$) and vertical (dose × time: $F(12,559) = 3.79$, $P < 0.001$) components of motor activity in non-habituated mice (Fig. 6). Both components of motor activity were significantly increased at high tested doses (30–60 mg/kg i.p.), and these effects were more pronounced in the second half of the test. Interestingly, none of the tested doses produced significant motor effects during the initial 15 min of the experiment. Again, the motor activity of mice habituated to their environment (Fig. 7) and injected with vehicle or ZM 241385 varied significantly across time, as indicated by the two-way ANOVA revealing an interaction between the time and dose factors in horizontal activity (dose × time: $F(12,215) = 10.80$, $P < 0.001$) and vertical activity (dose × time: $F(12,215) = 6.89$, $P < 0.001$). In habituated-mice, ZM 241385 (15–60 mg/kg i.p.) increased both horizontal and vertical activity only during the last three time sessions, with the effect being

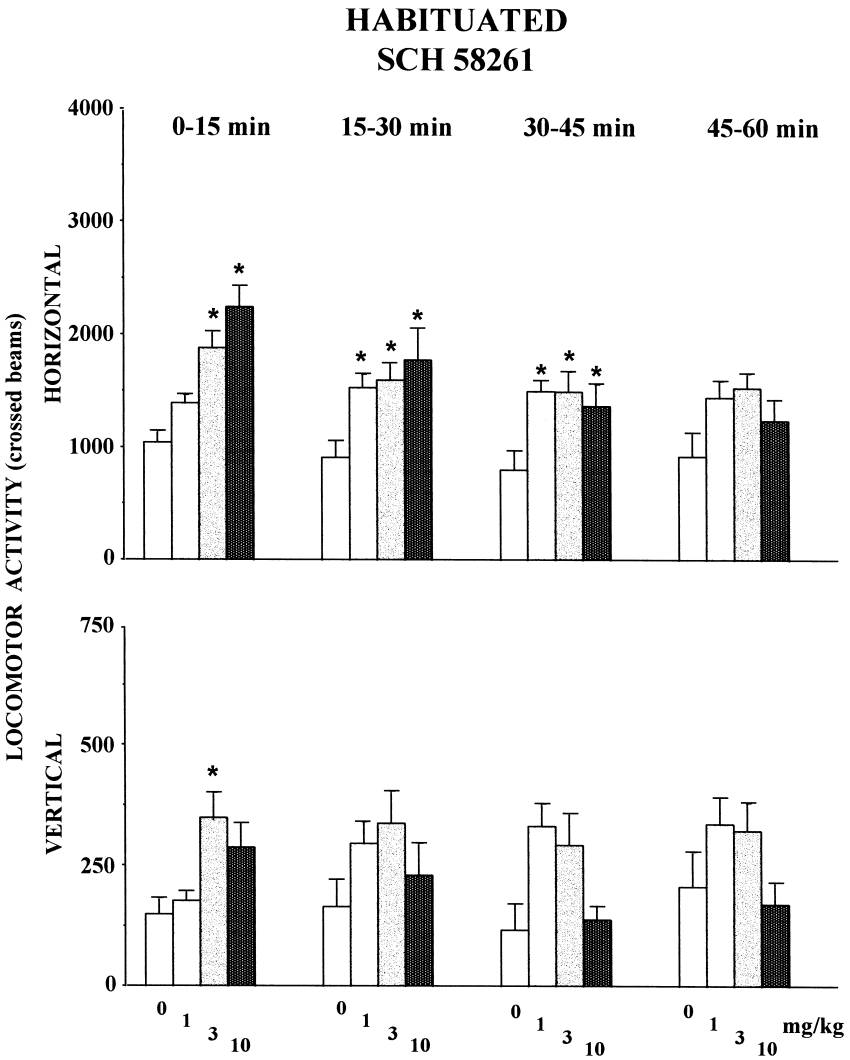

Fig. 5. Effects of the adenosine A_{2A} receptor antagonist SCH 58261 on motor activity in habituated mice. Mice were habituated to the test-cage for 30 min. They were then injected with vehicle (open bars) or increasing doses of SCH 58261 (doses and symbols are the same as in Fig. 4) and were returned to the actometers. The horizontal (upper panel) and vertical (lower panel) components of motor activity were measured for 60 min. Means ± S.E.M. of data from 13 mice/group. * $P < 0.05$ (by Newman–Keuls post hoc test following a one-way ANOVA).

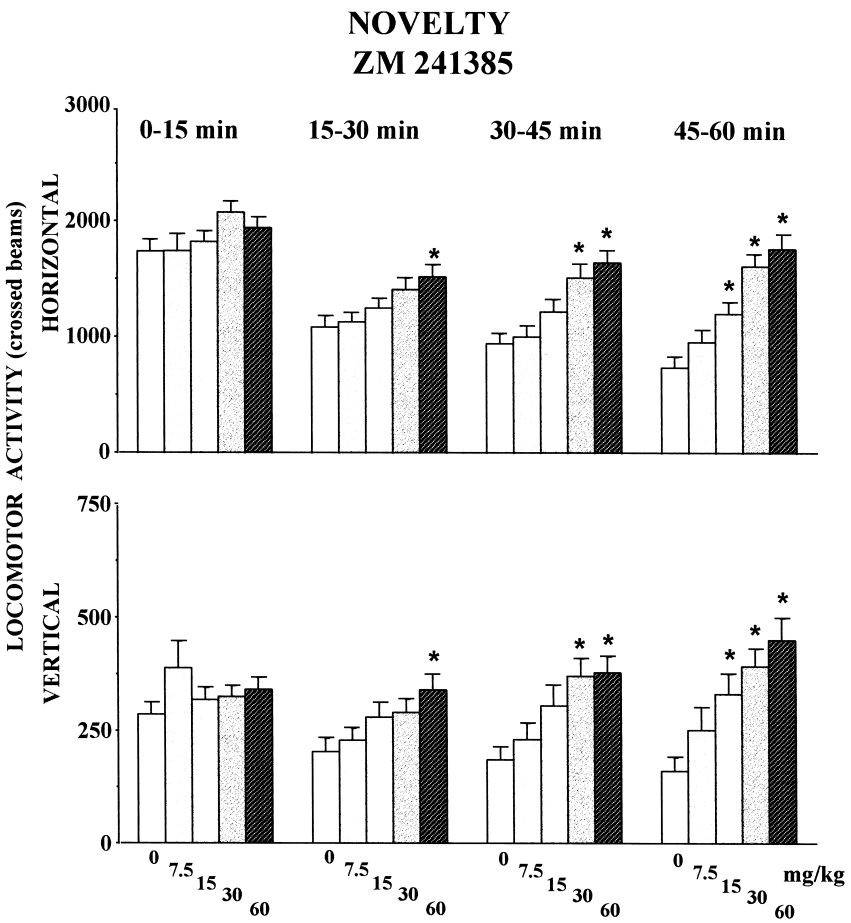

Fig. 6. Effects of the adenosine A_{2A} receptor antagonist ZM 241385 on motor activity in nonhabituated mice. Mice were injected with vehicle (open bars) or increasing doses of ZM 241385 (7.5–15–30–60 mg/kg i.p. hatched bars from grey to black) and were introduced into the actometers. The horizontal (upper panel) and vertical (lower panel) components of motor activity were measured for 60 min. Means ± S.E.M. of data from 30 controls and 20–30 mice in treated groups. $^{*}P < 0.05$ (by Newman–Keuls post hoc test following a one-way ANOVA).

greater in the last periods during which the activity associated with the stress of injection was substantially reduced in control group.

3.4. Comparison between SCH 58261 and ZM 241385

Figs. 4 and 6 show that time courses of novelty-induced motor activity in vehicle-treated mice were broadly similar in SCH 58261 and ZM 241385 experiments. However, as shown in Figs. 5 and 7, activity in habituated mice injected with vehicle displayed quite dissimilar time courses. The comparisons between Figs. 4–6 and Figs. 5–7 show that regardless of pharmacological treatments, the habituated mice demonstrated less horizontal activity than nonhabituated animals during the first 45 min (SCH 58261) or throughout the whole experiment (ZM 241385). Thus, the ANOVAs indicate a main effect of habituation, but no difference between the effects of SCH 58261 or ZM 241385 in the two conditions and no habituation × drug interaction except in one case. When the last 15-min period was considered, a significant interaction was indeed obtained between habituation and SCH 58261 factors [$F(3,117) = 3.86$, $P = 0.01$]. The levels of vertical activity differed between the novel open field situation vs. reexposure to it during the first half of the SCH 58261 experiment. During the last 0.5 h of the experiment, vertical activity was no longer influenced by the novelty factor. In the experiment with ZM 241385, the habituation factor influenced vertical activity counts during 45 min. A significant interaction occurred between habituation and ZM 241385 factors [$F(4,193) = 3.55$, $P < 0.01$] when the last 15-min period was considered.

Horizontal and vertical activities in habituated mice (Figs. 5–7) injected with vehicle displayed dissimilar time courses as evidenced by a Kruskal–Wallis one-way ANOVA on ranks: horizontal motor activity with $H = 9.95$, $P < 0.01$; vertical motor activity with $H = 7.65$; $P < 0.01$. To check whether this dissimilarity might have introduced artifacts in these experiments, an additional experiment was performed using the two adenosine A_{2A} receptor antagonists SCH 58261 and ZM 241385 in mice habituated to the open field for a longer (1 h) period. The results are shown in Table 1. In mice thoroughly habituated to their environment, SCH 58261 (1–10 mg/kg i.p.) also

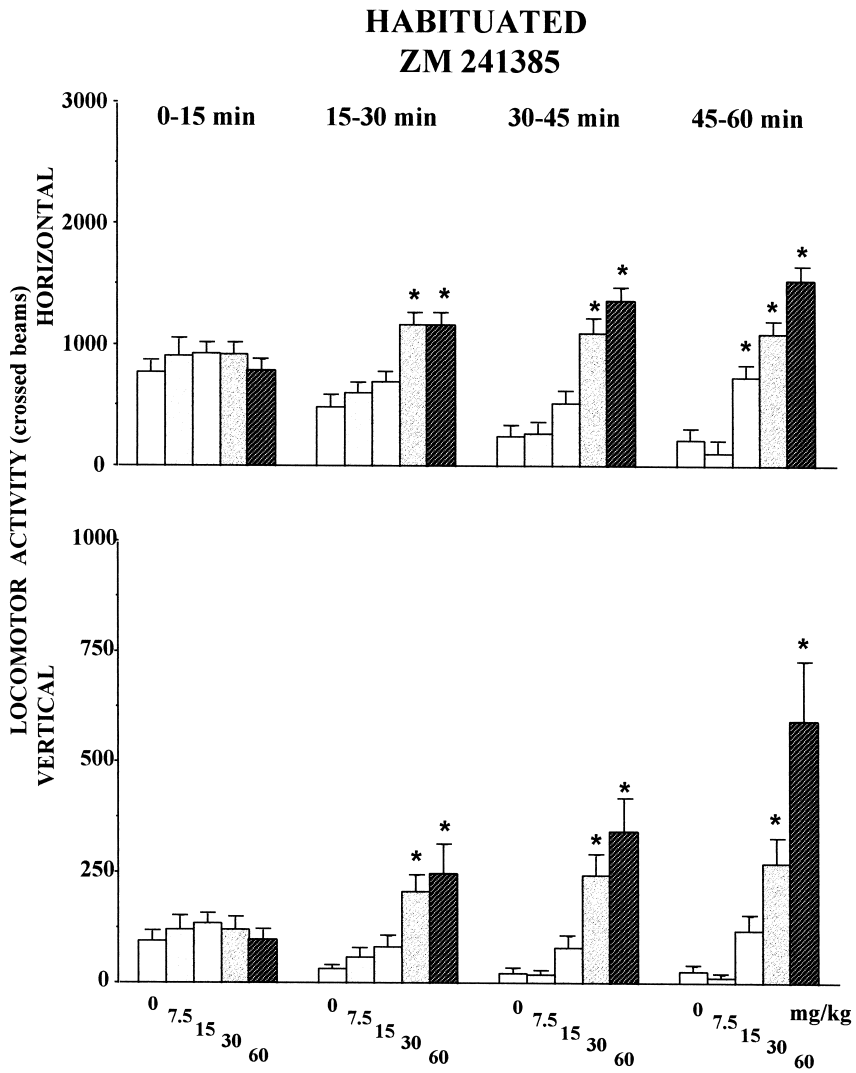

Fig. 7. Effects of the adenosine A_{2A} receptor antagonist ZM 241385 on motor activity in habituated mice. Mice were habituated to the test-cage for 30 min. They were then injected with vehicle (open bars) or increasing doses of ZM 241385 (doses and symbols are the same as in Fig. 6) and were introduced into the actometers. The horizontal (upper panel) and vertical (lower panel) components of motor activity were measured for 60 min. Means ± S.E.M. of data from 14 controls and 10 mice/group. * $P < 0.05$ (by Newman–Keuls post hoc test following a one-way ANOVA).

Table 1
Effects of increasing doses of SCH 58261 or ZM 241385 on motor activity in thoroughly habituated mice

Drugs	0–15 min		15–30 min		30–45 min		45–60 min	
	HLA	VLA	HLA	VLA	HLA	VLA	HLA	VLA
VEHICLE	1353 ± 102	245 ± 29	962 ± 107	155 ± 25	888 ± 121	180 ± 44	1010 ± 110	230 ± 47
SCH 58261 1 mg/kg	1357 ± 90	229 ± 37	1234 ± 111	258 ± 52	1178 ± 144	265 ± 61	1116 ± 194	282 ± 75
SCH 58261 10 mg/kg	2353[c] ± 166	294 ± 35	1434[a] ± 148	204 ± 37	1462[a] ± 131	265 ± 51	1476 ± 177	279 ± 60
ZM 241385 15 mg/kg	1086 ± 112	198 ± 47	1068 ± 150	259 ± 62	1180 ± 208	351[a] ± 83	1443[a] ± 235	476[a] ± 94
ZM 241385 60 mg/kg	1171 ± 107	229 ± 52	1601[c] ± 100	400[c] ± 41	1886[c] ± 133	516[c] ± 66	1744[b] ± 169	468[a] ± 75

Mice habituated for 1 h to the actometers were injected immediately before testing with vehicle or two doses of SCH 58261 (1–10 mg/kg i.p.) or ZM 241385 (15–60 mg/kg i.p). Horizontal (HLA) and vertical (VLA) components of motor activity (crossed beams) were recorded for 60 min.
Means ± S.E.M. of data from 18 controls and 13–14 mice in treated groups.
[a] $P < 0.05$ (by Newman–Keuls post hoc test following a two-way ANOVA with repeated measure).
[b] $P < 0.01$ (by Newman–Keuls post hoc test following a two-way ANOVA with repeated measure).
[c] $P < 0.001$ (by Newman–Keuls post hoc test following a two-way ANOVA with repeated measure).

time-dependently (dose × time: $F(6,126) = 3.12$, $P < 0.01$) increased horizontal activity, again only during the first 45 min of the experiment. Vertical activity was not modified in a time-dependent manner (dose × time: $F(6,126) = 0.84$, $P > 0.05$) and indeed SCH 58261 did not increase this component of motor activity in this experiment ($F(2,126) = 0.85$, $P > 0.05$). By contrast, thoroughly habituated mice receiving ZM 241385 (15–60 mg/kg i.p.) increased both horizontal (dose × time: $F(6,126) = 7.24$, $P < 0.001$) and vertical (dose × time: $F(6,126) = 5.57$, $P < 0.001$) activities in a time-dependent manner. These effects were obvious during the last 0.5 h of this experiment in which the activity associated with the stress of injection was very low in control group (Table 1).

3.5. Interaction with CGS 21680

3.5.1. Interaction after i.p. administration of SCH 58261 or ZM 241385

The effects of CGS 21680 on motor responses caused by SCH 58261 or ZM 241385 administered via the i.p. route in nonhabituated mice are shown in Fig. 8. CGS 21680 (0.5 mg/kg i.p.) was administered 15 min after SCH 58261 (1–3–10 mg/kg i.p.). There was no CGS 21680 × SCH 58261 interaction during this experiment for any of the two behavioural measures: horizontal activity, [$F(3,177) = 1.99$, $P > 0.05$]; vertical activity, [$F(3,177) = 0.81$, $P > 0.05$]. The analysis of the horizontal and vertical components of motor activity in these mice showed

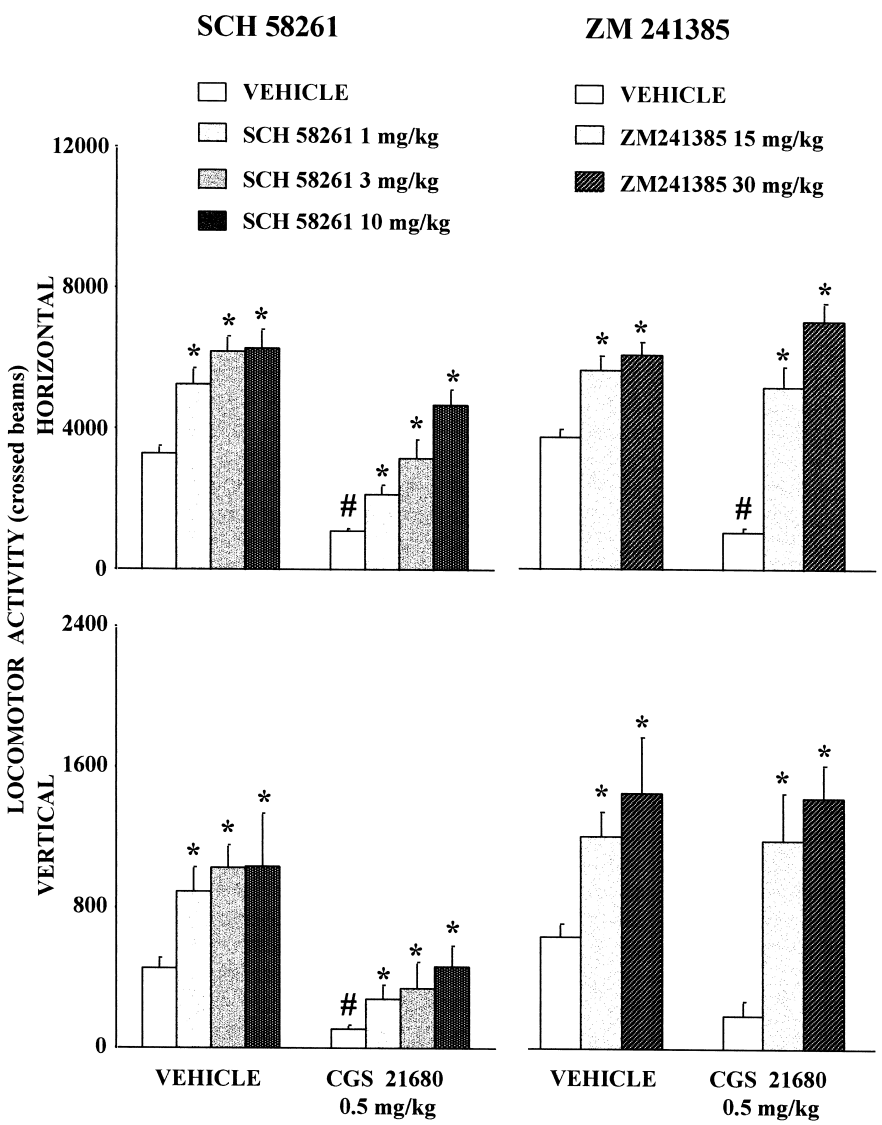

Fig. 8. Effects of SCH 58261 or ZM 241385 administered by i.p. route on inhibition of motor activity induced by CGS 21680 in nonhabituated mice. Mice were injected with vehicle (open bars), of SCH 58261 (1–3–10 mg/kg i.p. symbols as in Fig. 4) (left panel), ZM 241385 (15–30 mg/kg i.p. symbols as in Fig. 6) (right panel). Fifteen minutes later, they were injected with vehicle or CGS 21680 (0.5 mg/kg i.p.) and were introduced into the actometers. The horizontal (upper panel) and vertical (lower panel) components of motor activity were measured for 60 min. Means ± S.E.M. of data from 30–47 controls and 10–23 mice in treated groups. * $P < 0.001$ as compared with respective control groups; # $P < 0.001$ as compared with vehicle–vehicle injected group (by Newman–Keuls post hoc test following a two-way ANOVA).

main effects of CGS 21680 and SCH 58261 for both parameters: (a) CGS 21680 (horizontal activity, $[F(1,177) = 93.13,\ P < 0.001]$; vertical activity, $[F(1,177) = 45.80,\ P < 0.001)]$; (b) SCH 58261 (horizontal activity, $[F(3,177) = 30.17,\ P < 0.001]$; vertical activity, $[F(1,177) = 6.47,\ P < 0.001)]$. SCH 58261 significantly increased horizontal and vertical activities at each tested doses whether CGS 21680 was administered or not as indicated by post hoc tests (Fig. 8; left panel).

The overall two-way ANOVA of the horizontal component of motor activity revealed a significant interaction between CGS 21680 and ZM 241385 (15–30 mg/kg i.p.) treatments $[F(2,119) = 13.63,\ P < 0.001]$ in this experiment. Separate one-way ANOVAs were computed for the effects of ZM 241385 treatment on horizontal motor activity at each dose level of CGS 21680. Post hoc tests following the one-way ANOVA $[F(2,59) = 18.72,\ P < 0.001]$ indicated that the two doses of this adenosine A_{2A} receptor antagonist increased horizontal activity in vehicle-treated mice. In mice treated with CGS 21680 (0.5 mg/kg i.p.), the one-way ANOVA showed that ZM 241385 also stimulated significantly horizontal activity $[F(2,59) = 75.13,\ P < 0.001]$, and this effect was present at both tested doses (post hoc tests). The analysis of the vertical component of motor activity indicated that there was no CGS 21680 × ZM 241385 interaction concerning this behaviour $[F(2,119) = 1.08,\ P > 0.05]$. The decrease in rearing observed in vehicle pretreated mice did not reach a significant level $[F(2,119) = 1.50,\ P > 0.05]$. On the other hand, post hoc tests indicated that both doses of ZM 241385 still significantly increased rearing activity $[F(2,119) = 20.76,\ P < 0.001]$ in CGS 21680-treated mice (Fig. 8, right panel).

3.5.2. Interaction after i.c.v. administration of SCH 58261 or ZM 241385

The effects of CGS 21680 on motor responses caused by SCH 58261 or ZM 241385 administered directly into the brain of nonhabituated mice are shown in Fig. 9. CGS 21680 (0.5 mg/kg i.p.) was administered 15 min after SCH 58261 or ZM 241385 (5–10–20 nM i.c.v.). There was no CGS 21680 × SCH 58261 interaction during this experiment concerning horizontal activity, $[F(3,89) = 1.76,\ P > 0.05]$. The ANOVA showed main effects of CGS 21680 and SCH 58261: (a) CGS 21680 $[F(1,89) = 87.2,\ P < 0.001]$; (b) SCH 58261 $[F(3,89) = 3.96,\ P = 0.01]$. SCH 58261 significantly, but modestly, increased horizontal activity at two doses (10–20 nM) whether CGS 21680 was administered or not as indicated by post hoc tests (Fig. 9; upper panel). The overall two-way ANOVA of the horizontal component of motor activity did not either reveal an interaction between CGS 21680 and ZM 241385 (5–10–20 nM i.c.v.) treatments $[F(3,93) = 0.58,\ P > 0.05]$ in this experiment. The ANOVA showed main effects of CGS 21680 and ZM 241385: (a) CGS 21680 $[F(1,93) = 14.7,\ P < 0.001]$; (b) ZM 241385 $[F(3,93) = 13.64,\ P < 0.001]$. ZM 241385 induced a highly significant increase in horizontal activity at the two high doses (10–20 nM) whether CGS 21680 was administered or not as indicated by post hoc tests. This was clearly seen in CGS 21680-treated mice since motor activity counts were returned above control levels (Fig. 9; lower panel).

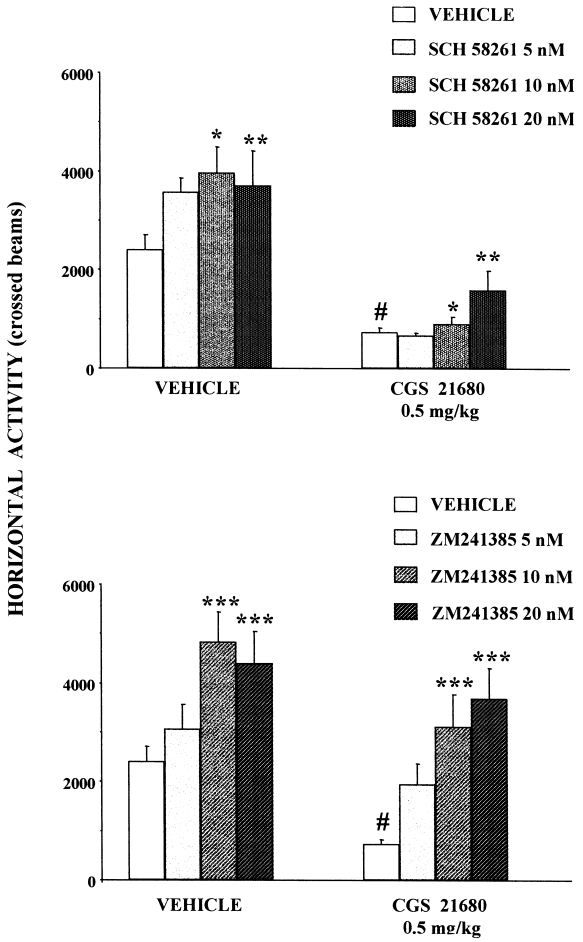

Fig. 9. Effects of SCH 58261 or ZM 241385 administered by i.c.v. route on inhibition of motor activity induced by CGS 21680 in nonhabituated mice. Mice were injected with vehicle (open bars), SCH 58261 (5–10–20 nM i.c.v.) (upper panel), ZM 241385 (5–10–20 nM i.c.v.) (lower panel). Fifteen minutes later, they were injected with vehicle or CGS 21680 (0.5 mg/kg i.p.) and were introduced into the actometers. The horizontal component of motor activity was measured for 60 min. Means ± S.E.M. of data from 18 controls and 9–13 mice in treated groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with respective control groups; $\#P < 0.001$ as compared with vehicle–vehicle injected group (by Newman–Keuls post hoc test following a two-way ANOVA).

Finally, the effects of acute SCH 58261 (10 mg/kg i.p.), administered alone or in combination with DPCPX (1 mg/kg), on the decrease in motor activity elicited by CGS 21680 (0.5 mg/kg i.p.) injected 15 min later are shown in Fig. 10. In this experiment, analysis of the effects of SCH 58261 and/or DPCPX pretreatments on effects elicited by CGS 21680 or its vehicle on motor activity yielded significant main effects of SCH 58261 administration $[F(1,72) = 44.92,\ P < 0.001]$ and CGS 21680 treatment $[F(1,72) =$

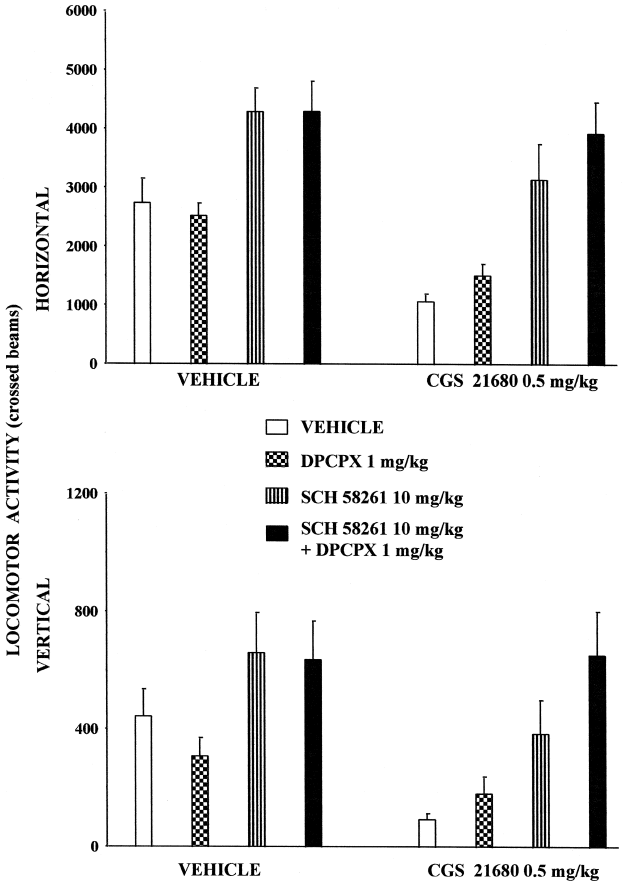

Fig. 10. Effects of SCH 58261, alone or in combination with DPCPX, on inhibition of motor activity induced by CGS 21680 in nonhabituated mice. Mice were injected with vehicle (open bars), SCH 58261 (10 mg/kg i.p.), DPCPX (1 mg/kg i.p.) or their combination. Fifteen minutes later, they received vehicle or CGS 21680 (0.5 mg/kg i.p.) and were introduced into the actometers. The horizontal (upper panel) and vertical (lower panel) components of motor activity were measured for 30 min. Means ± S.E.M. of data from 10 mice/group. A two-way ANOVA showed main effects of SCH 58261 and CGS 21680 upon both studied parameters.

12.97, $P < 0.001$], but no effect of DPCPX pretreatment upon horizontal activity [$F(1,72) = 0.76$, $P > 0.05$]. No significant interactions were found between SCH 58261 and CGS 21680 [$F(1,72) = 1.03$, $P > 0.05$], SCH 58261 and DPCPX [$F(1,72) = 0.23$, $P > 0.05$], CGS 21680 and DPCPX [$F(1,72) = 1.54$, $P > 0.05$]. There was no SCH 58261 × DPCPX × CGS 21680 interaction [$F(1,72) = 0.01$, $P > 0.05$] in the horizontal activity. Concerning the vertical activity parameter, the analysis revealed significant main effects of SCH 58261 [$F(1,72) = 19.74$, $P < 0.001$] and CGS 21680 treatment [$F(1,72) = 6.24$, $P < 0.05$], but no effect of DPCPX pretreatment [$F(1,72) = 0.45$, $P > 0.05$]. Again, no significant interactions were found between SCH 58261 and CGS 21680 [$F(1,72) = 0.55$, $P > 0.05$], SCH 58261 and DPCPX [$F(1,72) = 0.98$, $P > 0.05$], although the interaction between CGS 21680 and DPCPX factors approached the level of significance [$F(1,72) = 3.04$, $P = 0.08$]. There was no SCH 58261 × DPCPX × CGS 21680 interaction [$F(1,72) = 0.05$, $P > 0.05$] in this vertical component of motor activity.

4. Discussion

In the present study, we report that the adenosine A_{2A} receptor antagonists SCH 58261 and ZM 241385 exhibit a stimulant effect on motor activity in mice habituated or not to the motility cages. By contrast, the adenosine A_{2A} receptor agonist CGS 21680 inhibits motor activity by acting on centrally located adenosine A_{2A} receptors. Moreover, the pharmacokinetic and pharmacological profiles of the two antagonists are different. By analysis of the pharmacological profile of both adenosine receptor agonists and antagonists to alter motor activity in rodents, previous studies have led to the notion that activation of adenosine A_{2A} receptor plays a major role in the suppression of motor activity induced by adenosine (Phillis et al., 1986; Durcan and Morgan, 1989a; Nikodijevic et al., 1991). Under our experimental conditions, the selective adenosine A_{2A} receptor agonist CGS 21680 (Hutchinson et al., 1989; Jarvis et al., 1989) suppresses dose-dependently both ambulation and rearing of CD1 mice nonhabituated to the motility cages. The 0.5 mg/kg i.p. dose was chosen for further experiments as it reliably depressed both parameters in this pilot experiment. The same doses of CGS 21680 were needed to bring down horizontal (ambulation) and vertical (rearing) components of motor activity, as previously reported in rats (Rimondini et al., 1997). The present results, therefore, support previous findings showing that an adenosine A_{2A} receptor-mediated mechanism modulates distinct behaviours, respectively, in the dorsal striatum (rearing) and ventral striatum (ambulation) (Barraco and Bryant, 1987; Barraco et al., 1994). It could be argued that the equipotent effect of CGS 21680 on horizontal and vertical components of motor activity may be related to an unspecific general depressant effect of the drug due to, for instance, its peripheral side effects, like hypotension (Hutchinson et al., 1989; Casati et al., 1994; Alberti et al., 1997; Ledent et al., 1997). However, 8-SPT, a polar analogue of theophylline that does not cross the blood brain barrier (Evoniuk et al., 1987), was without significant effects upon CGS 21680-induced hypolocomotion at the doses used in the present study. Therefore, it is very likely that CGS 21680 exerts its depressant motor effects by stimulating central adenosine receptors. Previous studies also used similar doses of 8-SPT (Durcan and Morgan, 1989b; Nikodijevic et al., 1991; Marston et al., 1998) to prevent the peripheral cardiovascular effects of adenosine receptor agonists. As previously shown by Marston et al. (1998), 8-SPT itself was devoid of effect upon motor activity.

In two experimental paradigms different from the Digiscan actometer, the lack of effect of systemically administered CGS 21680 upon motor activity in adenosine A_{2A}

receptor knockout mice confirms our previous results (Ledent et al., 1997) showing that the adenosine A_{2A} receptor agonist completely looses its motor depressant effect in adenosine A_{2A} receptor knockout mice. This indicates that the adenosine A_{2A} receptor is probably the only target responsible for the behavioural effects elicited by a 0.5 mg/kg i.p. dose of CGS 21680 in the mouse.

The two adenosine A_{2A} receptor antagonists SCH 58261 (Zocchi et al., 1996) and ZM 241385 (Poucher et al., 1995; Keddie et al., 1996) exert dose-dependent motor stimulant effects in CD1 mice habituated or not to the environment. As already shown in other experiments performed in mice that used two other selective adenosine A_{2A} receptor antagonists, chlorostyrylcaffeine (Jacobson et al., 1993b) or (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine, i.e. KF 17837 (Marston et al., 1998), it is worth mentioning that the stimulant effect elicited by adenosine A_{2A} receptor antagonists is milder in intensity than that elicited by indirect dopamine receptor agonists, such as cocaine and amphetamine, for example (Vaugeois et al., 1993). Indeed, it has been clearly demonstrated that caffeine, which is a nonselective adenosine A_1/A_{2A} receptor antagonist, activates basal ganglia neurotransmission and thereby motor behaviour, in a mode different from that elicited by these psychostimulants (Svenningsson and Fredholm, 1997). Similar stimulant motor effect have also been obtained in rats with SCH 58261 (Svenningsson et al., 1997; Popoli et al., 1998).

The profiles of the motor effects elicited by SCH 58261 and ZM 241385 in mice are remarkably dissimilar. In nonhabituated mice, i.e. when administered to mice exploring a new environment, SCH 58261 increased ambulation and rearing immediately after injection for about 1 h. In habituated mice, which exhibit much lower levels of both ambulation and rearing, the effects of SCH 58261 were more transient. These findings provide a further indication upon the rapid penetration of this drug into the brain (Ongini, 1997). On the contrary, the higher doses of ZM 241385 used in the present study induced a time-dependent increase in ambulation and rearing with a slow onset indicating that this antagonist may enter slowly and maybe also to a limited extent into the brain. Thus, the different onset of behavioural effects seen between the two adenosine receptor antagonists is likely related to a pharmacokinetic mechanism.

It has been previously shown that the anxiogenic-like effects induced by caffeine in mice in several tests sensitive to stress-eliciting stimuli were not shared by the selective adenosine A_{2A} receptor antagonists used in the present study. Moreover, it was suggested that the differing motor effects elicited by high doses of caffeine in mice placed into a novel vs. a familiar environment might be related to its anxiogenic-like effects (El Yacoubi et al., 2000a). The present findings are in accordance with this previous work. Indeed, in contrast to caffeine, which decreased locomotion at high doses, the two selective adenosine A_{2A} receptor antagonists stimulated motor activity when placed into a novel environment that represents a stressful experimental condition (Belzung and Le Pape, 1994). Furthermore, the present data are in line with the suggestion that caffeine and other adenosine receptor antagonists increase wakefulness though the blockade of adenosine A_{2A} receptor (Bertorelli et al., 1996).

They also suggest that adenosine A_{2A} receptors located either in the dorsal striatum (rearing) or in the ventral striatum (ambulation) are tonically activated by endogenous adenosine and that adenosine A_{2A} receptor blockade may induce some functional effects independently of modulation of dopamine D_2 receptor transduction mechanisms, i.e. when dopamine exerts less tonic action upon dopamine D_2 receptor, as it has been previously suggested (Svenningsson et al., 1995, 1997).

If adenosine A_{2A} receptors are preferentially distributed to basal ganglia of the brain, there is also evidence for the presence of a small number of adenosine A_{2A} receptors in hippocampus and cortex (Ongini and Fredholm, 1996). In the hippocampus and cortex, brain areas known to be involved in arousal and in attentional processes (Acquas et al., 1996), a proportion of the specific binding of CGS 21680 corresponds to binding to typical ('striatal-like') adenosine A_{2A} receptors, whereas the remaining of the specific binding of CGS 21680 might represent binding to a hitherto unrecognised binding site (Johansson and Fredholm, 1995). The functional antagonism elicited by the two adenosine A_{2A} receptor antagonists towards the changes in motor activity induced by CGS 21680 was, therefore, investigated.

In vitro, both SCH 58261 and ZM 241385 display a similar subnanomolar affinity towards the human adenosine A_{2A} receptor and comparable A_{2A} vs. A_1 selectivity profiles. However, ZM 241385 has been reported to interact with human adenosine A_{2B} receptor, showing only a limited 60-fold selectivity for A_{2A} vs. A_{2B} selectivity (Ongini et al., 1999). Moreover, Lindström et al. (1996) using the in vitro quantitative autoradiography technique in rat brain showed that SCH 58261 is more than 1000-fold selective for 'striatal-type' adenosine A_{2A} receptors over 'atypical' cortical/hippocampal CGS 21680 binding sites. These 'atypical' sites would represent only about 15% of total specific CGS 21680 binding sites in the striatum (Cunha et al., 1996). On the other hand, Cunha et al. (1997) found that ZM 241385 was the first adenosine A_{2A} receptor antagonist with equal potency to displace tritiated CGS 21680 binding to striatal and hippocampal regions. Under our experimental conditions, the adenosine A_{2A} receptor antagonist ZM 241385 not only fully prevented the depressant locomotor effects induced by CGS 21680 when administered by the i.p. route, but retained a stimulant effect when directly administered into the brain to counteract CGS 21680-induced depressant effects. I.c.v. administration was used in order to get round the problem of interpretation of the results that might arise from the

different pharmacokinetic profiles of the two adenosine A_{2A} receptor antagonists. Indeed, the motor effects elicited by ZM 241385 (20 nM i.c.v.) in CGS 21680-treated mice were fivefold greater than those seen in CGS 21680-vehicle group and were above the level observed in vehicle–vehicle group. By contrast, even if SCH 58261 prevented in a dose-dependent manner the hypolocomotion induced by CGS 21680, it was unable to elicit stimulant effects in CGS 21680-treated mice of the same intensity level as in vehicle-treated controls. This was notably obvious after i.c.v. administration, as the locomotor activity counts following the highest dose (20 nM) of SCH 58261 were only twice that seen in animals receiving an i.c.v. injection of vehicle. It can be mentioned that the 10 mg/kg dose of SCH 58261 used here is a high dose as compared to the effective doses in a cerebral ischaemia model (Monopoli et al., 1998). The greater affinity of ZM 241385 towards the adenosine A_{2B} receptor might contribute to its better efficiency to counteract the decrease in motor activity elicited by CGS 21680. However, CGS 21680 has an extremely low affinity towards the adenosine A_{2B} receptor (Feoktistov and Biaggioni, 1997) and the complete loss of the hypolocomotor effects elicited by CGS 21680 in adenosine A_{2A} receptor knockout mice (Ledent et al., 1997; this study) strongly suggests an alternative explanation. One may speculate that the different affinity of the two antagonists towards the so-called 'atypical' CGS 21680 binding site (Cunha et al., 1997) could account for this difference. In the present study, we looked for a preliminary evidence in favour of this hypothesis. The selective adenosine A_{2A} receptor antagonist SCH 58261, which discriminates between the two types of CGS 21680 binding sites (Lindström et al., 1996), was co-administered with DPCPX, which has been described, apart from being a reference A_1 receptor antagonist (Bruns et al., 1987), as being a powerful displacer of tritiated CGS 21680 from its 'atypical' binding sites (Cunha et al., 1996). When DPCPX was co-administered with SCH 58261, CGS 21680-induced depressant motor effects were slightly more easily prevented than when SCH 58261 was administered alone, albeit the interaction between the two drugs did not reach a statistically significant level. It is worth noting that, in the study by Marston et al. (1998), the selective A_{2A} receptor antagonist KF 17837 (Kanda et al., 1994; Nonaka et al., 1994) reversed the motor depressant effects elicited by the selective adenosine A_{2A} receptor agonist 2-[(2-aminoethylamino) carbonylethyl-phenylethylamino]-5′-ethylcarboxamido adenosine (APEC) with a profile similar to that elicited by SCH 58261 against CGS 21680-induced hypolocomotion. These results would go nicely with what is known about the high and low potencies, respectively, of the selective adenosine A_{2A} receptor agonist (APEC) and antagonist (KF 17837) to displace CGS 21680 from its 'atypical' binding sites. However, more data needs to be obtained before considering that antagonism of motor depressant effect induced by CGS 21680 may represent a simple functional assay to screen drugs acting on this 'atypical' CGS 21680 binding site. First, the results obtained with DPCPX should be extended to other ligands that do not possess in vitro submicromolar affinity towards any adenosine receptor subtype. However, the results obtained in adenosine A_{2A} receptor knockout mice strongly suggest, as already mentioned, that CGS 21680 does not interact at the used dose with the A_1 receptor in vivo. Second, another possible explanation for the slight accentuation of the SCH 58261-induced effect by DPCPX could be related to an interaction between adenosine A_{2A} and A_1 receptors in vivo (see Nikodijevic et al., 1991; Jacobson et al., 1993b), so that adenosine A_{2A} and A_1 receptor blockade might induce synergistic effects upon motor activity. The results of the last experiment in vehicle-treated mice showing no interaction between SCH 58261 and DPCPX under the same experimental conditions, together with previous data demonstrating that DPCPX did not potentiate the stimulant effect elicited by SCH 58261 in naive mice are against this suggestion (El Yacoubi et al., 2000b). Thus, one is tempted to speculate that 'atypical' CGS 21680 binding sites are not tonically activated by endogenous adenosine in brain areas involved in the control of motor activity. Third, to our knowledge, most of the experimental findings related to these atypical CGS 21680 binding sites have been obtained with rat or guinea pig tissues (Sebastiao and Ribeiro, 1996). Since the pharmacological profile of agonists slightly differ at rat and human adenosine A_{2A} receptors (Kull et al., 1999), caution is needed before extrapolating results obtained in a different species. A fourth element that complicates the interpretation of the results is related to the magnitude of the effector response that depends not only on the diversity (A_{2A}, A_1, 'atypical' CGS 21680 binding site), localisation and relative density of receptors present on both sides of the synapses where the neurochemical messages are transmitted, but also on the diversity and amount of available G proteins present here, and finally upon the nature and activity of the effector enzyme or channel (Arslan et al., 1999). One critical feature of 'atypical' CGS 21680 binding sites is their ability to couple to G_i/G_o proteins, whereas 'striatal-like' adenosine A_{2A} receptors are coupled to G_s proteins (Cunha et al., 1999).

The growing knowledge of the characteristics of this 'atypical' CGS 21680 binding site will certainly open new avenues of research that may lead in the near future to a better understanding of the function of adenosine A_{2A} receptors in the brain and elsewhere. Whether the simple behavioural assay used in the present work might prove useful to develop drugs acting on 'atypical' CGS 21680 binding sites remains elusive. However, the present findings suggest that the 'atypical' CGS 21680 binding sites might represent 'true' adenosine A_{2A} receptors that have been deleted in mutant adenosine A_{2A} receptor knockout mice. These receptors would not only be able to couple to adenylate cyclase/cAMP transducing system but also to

other transducing systems or effectors, as suggested by other authors (Gubitz et al., 1996; Nörenberg et al., 1997; Cunha et al., 1999). Much work must be done to confirm this hypothesis and to elucidate the functions of this peculiar receptor in physiological and pathophysiological conditions.

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