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Effects of adenosine receptor agonists and antagonists on audiogenic seizure-sensible DBA/2 mice

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Abstract

We have studied the effects of selective and non-selective adenosine receptor agonists and antagonists in audiogenic-seizure-sensitive DBA/2 mice, an animal model of generalized reflex epilepsy. With the exception of the adenosine A_3 receptor agonist, N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine (IB-MECA), all the agonists studied prevented the development of audiogenic seizures in a dose-dependent manner. The ED_{50} values against the clonic phase of the audiogenic seizures were low, that is: 0.06 mg/kg, i.p., for the adenosine A_1 receptor agonist, 2-chloro-N^6-cyclopentyladenosine (CCPA), 0.02 and 0.03 mg/kg, i.p., for the adenosine A_{2A} receptor agonists, 2-(4-(2-carboxyethyl)-phenylamino)-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) and 2-hexynyl-5′-*N*-ethyl-carboxamidoadenosine (2-HE-NECA), and 0.7 mg/kg, i.p., for the adenosine A_1/A_3 receptor agonist, N^6-2-(4-aminophenyl)ethyladenosine (APNEA). Conversely, the non-selective agonist, *N*-ethyl-carboxamidoadenosine (NECA), was highly potent, the ED_{50} being 0.0005 mg/kg, i.p. In the absence of auditory stimulation, the adenosine receptor antagonists increased the incidence of both clonic and tonic seizures in DBA/2 mice. The ED_{50} values were: for caffeine, 207.5 mg/kg, i.p., for the adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 327.8 mg/kg i.p., for the adenosine A_{2A} receptor antagonists, 3,7-dimethyl-1-propylxanthine (DPMX), 86.7 mg/kg i.p., for the (*E*,18%–*Z*,82%)7-methyl-8-(3,4-dimethoxystyryl)-1,3-dipropylxanthine (KF 17837), 69.1 mg/kg i.p., and 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-(4,3-*c*)1,2,4-triazolo(1,5-*c*)-pyrimidine (SCH 58261), 321.8 mg/kg i.p. The rank order of convulsant potency in our epileptic model, following intracerebroventricular administration, was DPCPX > DMPX > 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC) > KF 17837 > Caffeine > SCH 58261 > 5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo(1,5-*c*)quinazoline (CGS 15943). Following a subconvulsant audiogenic stimulus of 83 dB, all adenosine receptor antagonists induced both tonic and clonic seizures. The ED_{50} values for such proconvulsant effects were: for caffeine 0.04 mg/kg, i.p., for the adenosine A_1 receptor antagonist, DPCPX, 5.84 mg/kg, i.p., for the adenosine A_{2A} receptor antagonists, DMPX, 0.02 mg/kg, i.p., CGS 15943, 0.29 mg/kg i.p., KF 17837, 0.57 mg/kg, i.p., CSC 0.12 mg/kg, i.p. and SCH 58261 0.07 mg/kg, i.p., respectively. These data suggest that stimulation of adenosine A_1 and A_{2A} receptors is involved in the suppression of seizures.

Keywords: Epilepsy; Adenosine; Adenosine receptor subtype; Adenosine receptor agonist; Adenosine receptor antagonist; Seizure audiogenic; DBA/2 mice

1. Introduction

Adenosine has potent neuromodulatory actions in the central nervous system (CNS) (Daval et al., 1991). These include depression of synaptic activity and inhibition of neurotransmitter release (Ribeiro, 1991; Cunha et al., 1994) and given its overall activity, adenosine has been proposed as an endogenous anticonvulsant (Dunwiddie, 1985). Studies with hippocampal slices have demonstrated potent anticonvulsant activity of adenosine and its analogs in these in vitro models of epilepsy (Dunwiddie, 1980; Ault and Wang, 1986). In vivo animal studies have also shown that systemically administered adenosine protects against audiogenic seizures in sensitive mice (Maitre et al., 1974). In addition, inhibition of adenosine reuptake retards the de-

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velopment and reduces the severity and duration of seizures in the amygdala-kindled rat (Dragunow et al., 1985). Moreover, administration of adenosine analogs produces anticonvulsant effects, while non-selective antagonists of adenosine receptors, such as the xanthines including caffeine, are proconvulsant, and at high dose they are convulsants (De Sarro et al., 1991, 1996b, 1997; Nehlig et al., 1992; Fredholm, 1995).

Adenosine acts through G protein-coupled receptors. There is evidence for four different adenosine receptors: adenosine A_1, A_{2A}, A_{2B} and A_3 receptors, all of which have been cloned from several mammalian species (Fredholm et al., 1994). The function of the adenosine A_1 receptor in the brain, under both physiological and altered conditions, has been much studied (Stone et al., 1995). Thus, single administration of adenosine A_1 receptor agonists ameliorates, or even prevents, seizures elicited by chemical and electrical stimuli in a wide range of animal models, while adenosine A_1 receptor-selective antagonists worsen convulsions (Von Lubitz et al., 1993; Zhang et al., 1994). Little is known, however, of the function of adenosine A_{2A} receptors because of a lack of selective receptor ligands, especially adenosine A_{2A} receptor antagonists (Ongini and Fredholm, 1996). The adenosine A_{2A} receptor agonist, N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine, antagonizes seizures induced by methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) (Klitgaard et al., 1993). Furthermore, Adami et al. (1995) reported that the adenosine A_{2A} receptor agonist, 2-hexynyl-5′-*N*-ethylcarboxamidoadenosine (2-HE-NECA), and the adenosine receptor non-selective agonist, 5′-*N*-ethylcarboxamidoadenosine (NECA), both reduce pentylentetrazole-induced seizures in the rat. However, the adenosine A_{2A} receptor agonist, CGS 21680, has only limited ability to antagonize bicuculline-induced seizures (Von Lubitz et al., 1993; Zhang et al., 1994).

As for adenosine A_{2A} receptor antagonists, a series of 8-styrylxanthine derivatives have been shown to have high affinity and good selectivity at the adenosine A_{2A} receptors, such as 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC) and (*E*,18%–*Z*,82%)7-methyl-8-(3,4-dimethoxystyryl)-1,3-dipropyl-xanthine (KF 17837) (Muller and Stein, 1996). Among non-xanthine compounds, 5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo(1,5-*c*)quinazoline (CGS 15943) (Williams et al., 1987) has long been used in in vitro and in vivo studies, though it has little or no adenosine A_{2A} vs. A_1 selectivity. Using CGS 15943 as a prototype, some new compounds have been synthesized and found to possess potent adenosine A_{2A} receptor antagonist properties and high adenosine A_{2A} vs. A_1 selectivity (Baraldi et al., 1994), such as 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-(4,3-*c*)1,2,4-triazolo(1,5-*c*)-pyrimidine (SCH 58261). As for adenosine A_3 receptor agonists, it is unclear whether stimulation of these receptors is able to antagonize convulsions. Recently, Von Lubitz et al. (1995b) demonstrated that N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine (IB-MECA), an adenosine A_3 receptor-selective agonist, is significantly protective against NMDA- and pentylentetrazole-induced seizures in mice. Furthermore, the adenosine A_1/A_3 receptor agonist, N^6-2-(4-aminophenyl)ethyladenosine (APNEA) (Jacobson et al., 1995), was able to enhance the anticonvulsant activity of phenytoin, phenobarbital and valproate (Borowicz et al., 1997).

Using the new, selective, compounds and in an attempt to understand more about the role of adenosine in mechanisms underlying seizures, we have studied the effects of selective and non-selective adenosine receptor agonists and antagonists in audiogenic seizure-prone DBA/2 mice, an animal model of generalized reflex epilepsy (Chapman et al., 1984; Seyfried and Glaser, 1985; Engstrom and Woodbury, 1988). Specifically, we have studied whether adenosine A_1, A_{2A}, and A_3 receptor agonists are involved in the anticonvulsant activity of adenosine. In addition, we have examined whether adenosine A_1 and A_{2A} receptor antagonists have proconvulsant activity.

2. Materials and methods

2.1. Animals

Male and female DBA/2 mice weighing 6–12 g (22–26 days old) were used (Charles River, Calco, Como, Italy). The animals were housed in groups of 8–10 under a 12-h light/dark cycle (lights on at 7:00 a.m.) with food and water available ad libitum. Procedures involving animals and their care were conducted in conformity with international and national law and policies.

2.2. Experimental design

DBA/2 mice were exposed to auditory stimulation 30 min following intraperitoneal (i.p.) administration of vehicle or adenosine receptor agonists and antagonists. Individual mice were placed under a hemispheric plexiglas dome (58 cm diameter) and 60 s was allowed for habituation and assessment of locomotor activity. The animals were exposed to a convulsant stimulus (12–16 kHz, 109 dB) or subconvulsant stimulus (83 dB, only for studies with antagonists). The stimulus was applied for 60 s or until tonic extension occurred. Seizure response, as previously reported (Chapman et al., 1987; De Sarro et al., 1994), was assessed on the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus, 4 = respiratory arrest. The maximum response was recorded for each animal. Mice were injected i.p. with various agonists (0.1 μg–10 mg/kg) and antagonists (0.01–600 mg/kg) dissolved in a 5% solution of carboxymethylcellulose (0.1 ml/10 g body weight), and were observed for 120 min. Each dose of compound was tested on 10 animals. Rectal temperature

Table 1
Effects of adenosine receptor agonists against the clonic and tonic phase of audiogenic seizures in DBA/2 mice

Compounds	Receptor involved	Range of doses (mg/kg)	ED_{50} values (95% confidence limits)	
			Clonic seizures	Tonic extension
CCPA	A_1	0.03–1	0.056 (0.041–0.075)	0.052 (0.040–0.067)
NECA	A_1/A_{2A}	0.0001–0.1	0.0005 (0.0003–0.001)	0.0003 (0.0002–0.0007)
CGS 21680	A_{2A}	0.01–1	0.024 (0.011–0.053)	0.018 (0.012–0.027)
2-HE-NECA	A_{2A}	0.01–1	0.028 (0.015–0.052)	0.016 (0.009–0.027)
APNEA	A_1/A_3	0.33–3.3	0.67 (0.58–0.78)	0.54 (0.41–0.71)
IB-MECA	A_3	0.03–10	>10[a]	>10[a]

Drugs were administered intraperitoneally 30 min before auditory stimulation. All data were calculated according to the method of Litchfield and Wilcoxon (1949).
[a]Not active up to 10 mg/kg for IB-MECA.

was recorded immediately prior to the auditory testing using an Elektrolaboratoriet thermometer type T.E.3. Behavioral changes were evaluated during the period between drug administration and auditory testing. Latency to the onset of various phases of audiogenic seizures and incidence of each phase of seizures were recorded.

For intracerebroventricular (i.c.v.) injections, the mice were anesthetized with ether and injections were made in the left or right lateral ventricle (coordinates: 1 mm posterior and 1 mm lateral to the bregma; depth: 2.4 mm) using a 10-μl Hamilton microsyringe (type 701N) fitted with a nylon cuff on the needle as described elsewhere (De Sarro et al., 1988). Injection of vehicle (phosphate buffer) or drugs by this procedure leads to uniform distribution throughout the ventricular system within 10 min (De Sarro et al., 1994). The occurrence of clonic and tonic seizures and their latency were recorded.

2.3. Statistics

Statistical comparisons among groups of control and drug-treated animals were made using Fisher's exact probability test (incidence of the seizure phases) or analysis of variance (ANOVA) and Dunnett's *t*-test (rectal temperatures). The percentage incidence of each phase of the audiogenic seizure was determined for each dose of compound administered. These values were plotted against the corresponding doses by a computer construction of the curves for the calculation of ED_{50} values (with 95% confidence limits). ED_{50} values for each compound were estimated using a computer program of the method of Litchfield and Wilcoxon (1949). At least 32 animals were used for the calculation of each ED_{50} value.

2.4. Drugs

The following adenosine receptor agonists were purchased from Research Biochemicals (Natick, MA, USA): 2-chloro-N^6-cyclopentyladenosine (CCPA), 2-hexynyl-5′-*N*-ethylcarboxamidoadenosine (2-HE-NECA), 5′-*N*-ethylcarboxamidoadenosine (NECA), 2-(4-(2-carboxyethyl)-phenylamino)-5′-*N*-ethylcarboxamidoadenosine (CGS 21680), N^6-2-(4-aminophenyl)-ethyl-adenosine (APNEA), and N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine (IB-MECA). The following adenosine receptor antagonists were purchased from Research Biochemicals: 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, molecular weight (m.w.): 305.4), 3,7-dimethyl-1-propylxanthine (DMPX,

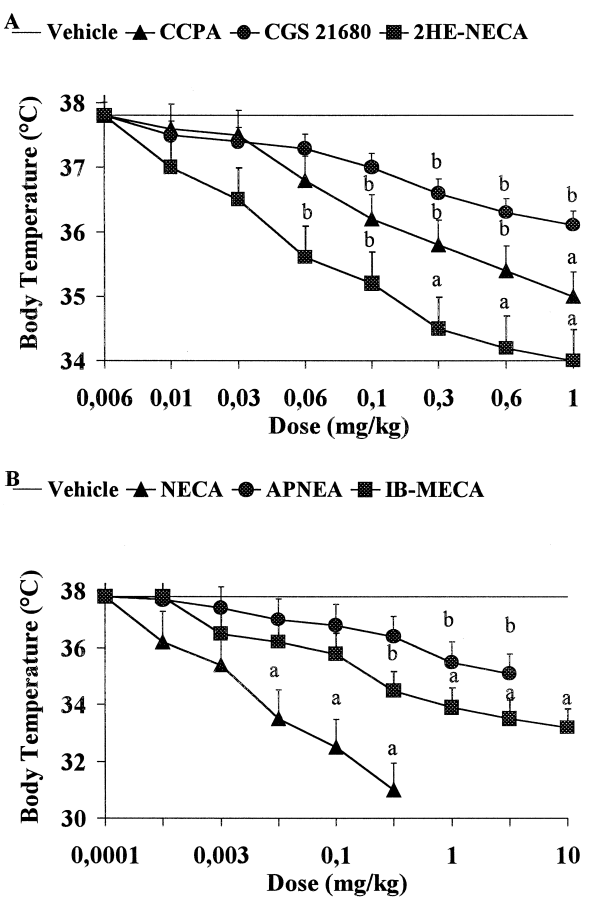

Fig. 1. Effects of various doses of adenosine receptor agonists on body temperature in DBA/2 mice 30 min after drug injection. Significant differences in decrease of body temperature between control and drug-treated group were as follows: (a) $P < 0.01$, (b) $P < 0.05$; ANOVA and Dunnett's *t*-test.

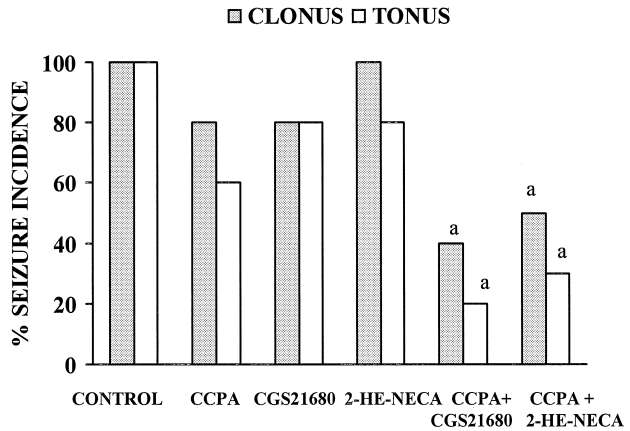

Fig. 2. Effects of subthreshold doses of adenosine receptor agonists in DBA/2 mice subjected to auditory stimulation. CCPA was injected at the dose of 0.04 mg/kg, while CGS 21680 and 2-HE-NECA were administered at the dose of 0.01 mg/kg, alone or in combination, and exposed to auditory stimulation 30 min after drug injection. Significant differences in the incidence of seizure phases between concurrent control and drug-treated group were as follows: (a) $P < 0.01$, (b) $P < 0.05$; Fisher's exact probability test.

m.w.: 218.22), 5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo(1,5-*c*)quinazoline (CGS 15943, m.w.: 285.69), 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC, m.w.: 330.78) and (*E*,18%–*Z*,82%)7-methyl-8-(3,4-dimethoxystyryl)-1,3-dipropylxanthine (KF 17837, m.w.:274.2) while caffeine (m.w.: 194.2) was purchased from Sigma (St. Louis, MO, USA). The adenosine A_{2A} receptor-selective antagonist, 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-(4,3-*c*)1,2,4-triazolo(1,5-*c*)-pyrimidine (SCH 58261, m.w.: 345.4), was from the Schering-Plough Research Institute (Milan, Italy).

3. Results

3.1. Adenosine receptor agonists

Adenosine receptor agonists acting preferentially at adenosine A_1 or A_{2A} receptors, given i.p. 30 min before the auditory stimulation, reduced the severity of the audiogenic seizures in DBA/2 mice. The ED_{50} values against clonic and tonic phases of the audiogenic seizures are reported in Table 1. With the adenosine A_1 receptor agonist, CCPA (0.03–1 mg/kg), and the adenosine receptor-non-selective agonist, NECA (0.0001–0.1 mg/kg), a dose-dependent reduction of the seizures was observed. Likewise, the adenosine A_{2A} receptor agonists, 2-HE-NECA (0.01–1 mg/kg) and CGS 21680 (0.01–1 mg/kg), reduced in a dose-dependent manner the incidence of both clonic and tonic extension seizures. The mixed adenosine A_1/A_3 receptor agonist, APNEA (0.33–3.3 mg/kg), dose dependently reduced the audiogenic seizures in DBA/2 mice, while the adenosine A_3 receptor agonist, IB-MECA (0.03–10 mg/kg), failed to do so. The wild running phase was significantly reduced after administration of all adenosine receptor agonists at the highest doses tested, with the exception of IB-MECA (data not shown). A significant fall in body temperature ($P < 0.01$) was observed after CCPA (0.1–1 mg/kg), NECA (0.03–0.33 mg/kg), 2-HE-NECA (0.06–1 mg/kg), CGS 21680 (0.3–1 mg/kg), APNEA (1–3 mg/kg) and IB-MECA (0.3–10 mg/kg) (Fig. 1).

Based on the ED_{50} values of the agonist compounds tested, NECA was 50, 100, and 1000-fold more potent to counteract the audiogenic seizures developed in DBA/2 mice, than adenosine A_{2A}, A_1 and A_3 receptor-selective agonists, respectively. Low doses of CCPA (0.04 mg/kg) and CGS 21680 (0.01 mg/kg) did not reduce the incidence and the severity of audiogenic seizures when administered 30 min before auditory stimulation. However, when they were administered simultaneously at such non-effective doses, they produced a marked reduction of both clonic and tonic phases (Fig. 2). Similarly, the concomitant administration of CCPA and 2-HE-NECA resulted in protection against tonic seizures (Fig. 2).

3.2. Adenosine receptor antagonists

As all DBA/2 mice display convulsions at first (data not shown), during elevated auditory stimulation (109 dB) we studied the effects of adenosine receptor antagonists in the absence of auditory stimulation. The non-selective adenosine receptor antagonist, caffeine, administered over a wide range of doses (50–300 mg/kg, i.p.), induced both clonic and tonic seizures (Table 2). The convulsive re-

Table 2
Effects of adenosine receptor antagonists in DBA/2 mice in the absence of auditory stimulation

Compounds	Receptor involved	Range of doses (mg/kg)	ED_{50} values (95% confidence limits)	
			Clonic seizures	Tonic extension
DPCPX	A_1	50–600	327.8 (255.1–421.1)	–
Caffeine	A_1/A_{2A}	50–300	207.5 (160.6–268.1)	292.6 (242.5–353.1)
DMPX	A_{2A}	50–300	86.7 (53.8–139.9)	123.9 (84.7–181.3)
KF 17837	A_{2A}	10–200	69.1 (48.5–98.4)	89.1 (55.3–143.6)
SCH 58261	A_{2A}	10–400	321.8 (291.6–355.2)	352.1 (318.2–389.6)

Drugs were administered intraperitoneally. The animals were observed for seizure onset in the absence of any auditory stimulation. All data were calculated according to the method of Litchfield and Wilcoxon (1949).

Table 3
Convulsant activity of various adenosine receptor antagonists in DBA/2 mice

Compounds	Range of doses (nmol/mouse)	Adenosine A_1/A_{2A} ratio	Clonic phase
DPCPX	(10–150)	0.002[a]	66.3 (34.4–127.8)
CGS 15943	(100–300)	5.3[a]	248 (227–271)
KF 17837	(50–300)	49[b]	171 (136–215)
SCH 58261	(10–400)	52.6[a]	235.1 (144.1–383.2)
Caffeine	(50–300)	0.6[c]	207.5 (160.6–268.1)
CSC	(50–300)	522[d]	93.2 (63.3–137.3)
DMPX	(50–300)	4.1[e]	86.7 (53.8–139.9)

Drugs were injected intracerebroventricularly. ED_{50} values (with 95% confidence limits) for the clonic and tonic phases of the audiogenic seizures are expressed as nmol/mouse and were calculated according to the method of Litchfield and Wilcoxon (1949).
Taken from [a](Zocchi et al., 1996); [b](Dionisotti et al., 1994); [c](Virus et al., 1990); [d](Jacobson et al., 1993); [e](Ukena et al., 1986).

sponse to caffeine in DBA/2 mice consisted of wild running, clonic seizures and loss of the righting reflex within 10 min after the i.p. administration of high doses of the drug. Similar effects were found after administration of the adenosine A_1 receptor antagonist, DPCPX (50–600 mg/kg), the adenosine A_{2A} receptor antagonists, DMPX (50–300 mg/kg), KF 17837 (10–200 mg/kg) and SCH 58261 (10–400 mg/kg) (Table 2). Lethality was observed after administration of DMPX, caffeine and KF 17837 at the high doses tested, while DPCPX and SCH 58261 did not cause mortality at any dose.

To overcome possible problems with blood–brain penetration we also studied the effects of DPCPX, CGS 15943, KF 17837, DMPX, CSC and SCH 58261 after i.c.v. administration. Thus, the adenosine A_1 receptor antagonist, DPCPX (25–150 nmol/mouse), produced a typical sequence of epileptic signs. In particular, 3–10 min following the injection, clonus of both fore- and hind-limbs was evident. These signs were usually followed by barrel rolling or wet-dog shake episodes. At the highest doses, the animals showed hindlimb tonic extension followed by death of three out of six mice. The ED_{50} value ($\pm$95% confidence limits) of DPCPX for clonus was 66.3 (34.4–127.8) nmol/mouse. The i.c.v. injection of adenosine A_{2A} receptor antagonists, CSC (50–300 nmol/mouse) and SCH 58261 (10–400 nmol/mouse), induced epileptic seizures similar to those observed after DPCPX. In addition, both CSC and SCH 58261 induced an increase of locomotor activity and some circling episodes, which usually appeared contralaterally to the site of microinjection. The ED_{50} values ($\pm$95% confidence limits) for CSC for clonus was 93.2 (63.3–137.3) and that of SCH 58261 was 235.1 (144.1–383.2) nmol/mouse. The ED_{50} values ($\pm$95% confidence limits) for caffeine, CGS 15943, KF 17837, DMPX are reported in Table 3. The order of convulsant potency in DBA/2 mice, following intracerebroventricular administration, was DPCPX > DMPX > CSC > KF 17837 > Caffeine > SCH 58261 > CGS 15943 (Table 3).

Additional studies were made with animals exposed to a subthreshold sound exposure of 83 dB, instead of 109 dB. Control mice did not develop seizures, while all the adenosine receptor antagonists studied facilitated in a dose-dependent manner the effects of the subthreshold sound exposure. In fact, following a subconvulsant audiogenic stimulus of 83 dB all the adenosine receptor antagonists induced seizures. The ED_{50} values for tonus and clonus are reported in Table 4.

3.3. Effects of DPCPX and CSC on anticonvulsant activity of CCPA and 2-HE-NECA

The concomitant treatment with DPCPX (20 nmol/mouse i.c.v.) was able to significantly increase, 2.7–3.2 times, the ED_{50} values for CCPA against clonus and tonus while the same treatment was able to increase

Table 4
Pro-convulsant activity of various adenosine receptor antagonists in DBA/2 mice after administration of a subconvulsant sound stimulus (83 dB)

Compounds	Receptor involved	Range of doses (mg/kg)	ED_{50} values (95% confidence limits)	
			Clonic seizures	Tonic extension
DPCPX	A_1	0.1–20	5.84 (1.30–23.14)	7.03 (2.42–20.38)
Caffeine	A_1/A_{2A}	0.01–0.3	0.04 (0.02–0.07)	0.06 (0.04–0.09)
CGS 15943	A_1/A_{2A}	0.1–10	0.29 (0.08–0.98)	1.47 (0.62–3.47)
DMPX	A_{2A}	0.01–1.0	0.02 (0.01–0.04)	0.04 (0.03–0.08)
KF 17837	A_{2A}	0.1–10	0.57 (0.31–1.04)	0.85 (0.52–1.39)
CSC	A_{2A}	0.033–5	0.12 (0.05–0.29)	0.30 (0.15–0.62)
SCH 58261	A_{2A}	0.01–3.3	0.07 (0.04–0.15)	0.09 (0.04–0.2)

Drugs were administered intraperitoneally 30 min before auditory stimulation. All data were calculated according to the method of Litchfield and Wilcoxon (1949).

Table 5
The effects of DPCPX on the anticonvulsant activity of CCPA and 2-HE-NECA against sound-induced seizures in DBA/2 mice

Treatment	ED_{50} values (95% confidence limits)	
	Clonic seizures	Tonic extension
CCPA + vehicle	0.056 (0.041–0.075)	0.052 (0.040–0.067)
CCPA + DPCPX	0.18 (0.11–0.24)[a]	0.14 (0.09–0.24)[a]
2-HE-NECA + vehicle	0.028 (0.015–0.052)	0.016 (0.009–0.027)
2-HE-NECA + DPCPX	0.043 (0.027–0.068)	0.018 (0.009–0.036)
CCPA + CSC	0.078 (0.055–0.111)	0.052 (0.023–0.117)
2-HE-NECA + CSC	0.082 (0.035–0.192)[a]	0.055 (0.036–0.084)[a]

CCPA and 2-HE-NECA were administered intraperitoneally 30 min before auditory stimulation, whereas vehicle, DPCPX (20 nmol/mouse) or CSC (25 nmol/mouse) were injected intracerebroventricularly 5 min after CCPA or 2-HE-NECA. All data were calculated according to the method of Litchfield and Wilcoxon (1949). [a]Denotes significant differences between concurrent groups at $P < 0.01$.

1.5 times the ED_{50} value for 2-HE-NECA against clonus (Table 5). In addition, the concomitant treatment with CSC (25 nmol/mouse i.c.v.) was able to significantly increase, 2.9–3.7 times, the ED_{50} values for 2-HE-NECA against clonus and tonus while the same treatment was able to increase 1.4 times the ED_{50} value for CCPA against clonus (Table 5).

4. Discussion

We have described the effects of adenosine receptor-selective agonists and antagonists in a genetic model of seizures, DBA/2 mice. The present results indicate that stimulation of either adenosine A_1 or A_{2A} receptors results in protection against audiogenic seizures. These data, together with other findings (Adami et al., 1995), suggest that both adenosine A_1 and A_{2A} receptors are involved in the suppression of seizures. Furthermore, we have demonstrated that concomitant administration of low doses of adenosine A_1 and A_{2A} receptor agonists, not active on their own, exerts a protective effect in this model, suggesting some interaction between the two types of adenosine receptors. Conversely, the adenosine A_3 receptor agonist, IB-MECA, has been found ineffective to block the development of audiogenic seizures. These latter results differ somewhat from those of Von Lubitz et al. (1995b), who showed that the adenosine A_3 receptor agonist, IB-MECA, was effective against NMDA- and pentylenetetrazole-induced seizures, but did not antagonize tonic convulsions produced by electroshock. The partial discrepancy of these results could be accounted for by the different mechanisms underlying seizures, i.e., NMDA- and pentylenetetrazole-induced seizures instead of electroshock or a genetic model, where chemical stimulation is not required. In addition, possible pharmacokinetic interactions must be considered. It has been recently suggested (Borowicz et al., 1997) that IB-MECA possesses vasocostricting properties which may produce a decrease in absorption of NMDA and pentylenetetrazole, a reduction in plasma levels and in brain penetration, so that the brain concentrations of these chemoconvulsants could not reach epileptogenic levels.

Adenosine is involved in the modulation of seizures and several findings support the notion that adenosine is an endogenous anticonvulsant (Dragunow et al., 1985). Immediately after the onset of seizures, adenosine levels in the brain rise rapidly and its levels have also been shown to remain elevated post-ictally and this may be critical in preventing further seizure development and/or propagation (During and Spencer, 1992; Young and Dragunow, 1994). Thus, stimulation of adenosine receptors reduced convulsions induced by a variety of chemical and electrical stimuli in a wide range of in vitro and in vivo models (De Sarro et al., 1991; During and Spencer, 1992). The anticonvulsant activity of adenosine analogs has been attributed to specific stimulation of the A_1 receptor subtype (Sanders and Murray, 1989; Von Lubitz et al., 1993; Zhang et al., 1993). This may occur through opening K^+ channels which in turn induce hyperpolarizing effects and reduction in excitability of postsynaptic neurons. Until recently, the adenosine A_{2A} receptors have received little attention for their role in the modulation of seizures. There is, however, one report showing that the adenosine A_{2A} receptor agonist, 2-HE-NECA, markedly reduces pentylentetrazole-induced lethal seizures (Adami et al., 1995). There are also data showing that stimulation of adenosine A_{2A} receptors by metrifudil, *N*-(2-methylphenyl)methyladenosine, and N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]-adenosine, reduces seizures induced by the convulsive β-carboline, DMCM, but these compounds interact with adenosine A_1 receptors as well (Klitgaard et al., 1993). There are contrasting results with CGS 21680, which appears either not to be effective or to have weak activity for the antagonism of bicuculline-induced seizures (Von Lubitz et al., 1993; Zhang et al., 1994). It is worth noting however that CGS 21680 penetrates poorly into the brain (Nikodijevic et al., 1990). In the present study we have demonstrated that the anticonvulsant properties of CCPA and 2-HE-NECA were reversed by adenosine A_1 or A_{2A} receptor-selective antagonists, i.e., DPCPX or CSC (Table 5).

It is hard to explain why selective stimulation of either adenosine A_1 or A_{2A} receptors can lead to the same net effect. The anticonvulsant properties of adenosine A_1 receptor agonists are attributed to the general inhibitory actions of adenosine, which are thought to occur through adenosine A_1 receptors (Von Lubitz et al., 1995a). Conversely, because of the possible role of adenosine A_{2A} receptors in mediating excitatory actions of adenosine in discrete brain areas such as the hippocampus, it is not expected that adenosine A_{2A} receptor agonists are effective to prevent seizures. Like adenosine A_1 receptors, adenosine A_{2A} receptors may modulate some of the post-

ictal depression observed after convulsions (Janusz and Berman, 1992). Since post-ictal depression represents a period of inhibitory activity that limits the spread and generalization of the seizure, this suggests that adenosine A_{2A} receptor activation may have some anticonvulsant effects. Given the distribution and functional role of adenosine A_{2A} receptors in non-neuronal tissues, such as cerebral vessels, the net anticonvulsant effect observed in vivo may well depend on a complex circuitry of interaction. Clearly, neurophysiological studies on specific neuronal cell preparations are needed to clarify whether adenosine A_{2A} receptors have a specific role in the regulating mechanisms underlying seizures. Despite their activity in a variety of models, adenosine receptor agonists are not potential antiepileptic agents because of the variety of peripheral, mainly cardiovascular, effects associated with this central action (Dunwiddie and Worth, 1982; Monopoli et al., 1994). Adenosine agonists significantly reduce body temperature in DBA/2 mice, and Bowker and Chapman (1986) demonstrated a clear correlation between changes in body temperature and inhibition of seizures. In fact, the latter authors showed that if the mice were warmed to prevent a fall in temperature, then the anticonvulsant activity was also prevented. We also found that if we prevented the fall in body temperature, the animals pretreated with NECA and adenosine A_1 receptor agonists were not protected against audiogenic seizures, while following adenosine A_2 receptor agonists a reduced anticonvulsant activity was still evident in warmed animals (data not shown). In addition, IB-MECA, an adenosine A_3 receptor agonist, shows hypothermic effects without anticonvulsant properties. We can conclude that the fall in body temperature might play a significant role in anticonvulsant effects of adenosine A_1 receptor agonists against audiogenic seizures. The present studies help, however, to clarify the mechanisms governing the different actions of the adenosine A_1 receptor compared to those of the adenosine A_{2A} receptor in the CNS.

In the present study, blockade of the adenosine receptors led to both clonic and tonic convulsant activity in the absence of audiogenic stimuli with either i.p. or i.c.v. administration route. However, the severity of seizures was largely dependent on the individual drug and occurred only at high doses. Thus, caffeine induced convulsions with clonic ED_{50} of 207 mg/kg, i.p., while the adenosine A_1 receptor antagonist, DPCPX, and the adenosine A_{2A} antagonists, KF 17837 and SCH 58261, produced convulsions with ED_{50}, for the clonic phase, of 328 mg/kg, i.p., 69 mg/kg, i.p., and 322 mg/kg, i.p., respectively. In order to make a more accurate comparison of the involvement of adenosine A_1 and A_{2A} receptors in convulsant properties of the xanthines, we administered the adenosine A_1 receptor antagonist, DPCPX, and the adenosine A_{2A} antagonists, CSC, CGS 15943, KF 17837, DMPX and SCH 58261, by i.c.v. injection. This route was utilized to reduce penetration caused differences in the CNS and in the distribution and rate of metabolism of these selective antagonists. The order of convulsant potency in DBA/2 mice, following intracerebroventricular administration, was DPCPX > DMPX > CSC > KF 17837 > Caffeine > SCH 58261 > CGS 15943 (Table 3). DPCPX was thus 3.5-fold more potent than SCH 58261 to elicit seizures, suggesting that both receptors are involved in the regulation of seizures, but that adenosine A_1 receptors probably play a predominant role. Recently, Ledent et al. (1997) have found that mice lacking the adenosine A_{2A} receptor showed various altered behavioral responses compared with those of wild-type animals, but did not develop seizures. No data are available for adenosine A_3 receptor antagonists, since compounds acting on this receptor have only recently been described (Karton et al., 1996; Van Rhee et al., 1996).

To assess whether blockade of adenosine receptors enhanced the susceptibility to seizures, we administered adenosine receptor antagonists before a subconvulsant audiogenic stimulus (83 dB). Again, we found different responses to individual drugs and to the doses used (Table 4). The latter results are difficult to reconcile with the blockade of a specific receptor (e.g., adenosine A_1 vs. A_{2A}) or to the potency of each drug. Furthermore, different brain penetration could determine a specific profile for each drug used and explain their different activities. Although the systemic administration of adenosine receptor antagonists has been widely shown to induce behavioral changes (Kanda et al., 1994; Bertorelli et al., 1996; De Sarro et al., 1997; Fenu et al., 1997), it is unclear to what extent they pass through the blood–brain barrier and act directly on central neurons. Unfortunately, our results do not help to resolve this issue and further investigation of the blood–brain penetration of these compounds would be useful to clarify the issue. The tendency to augment susceptibility to seizures is a common denominator in blockade of adenosine receptors. The methylxanthines are known to cause spontaneous seizures in various animal species, including humans, after high-dose exposure (Zwillich et al., 1975; Chu, 1981; Walker, 1981; De Sarro et al., 1997). At lower doses, they have been shown to prolong the after-discharge duration of kindled seizures without affecting the threshold of seizure beginning (Albertson et al., 1983; Dragunow et al., 1985; Dragunow and Robertson, 1987). It is important to note that DBA/2 mice are very sensitive and this is a particular animal model, i.e., the animals are prone to epilepsy, and for this reason it is important to compare the data from this model with results obtained from a battery of chemical convulsant tests before concluding that there is a true proconvulsant drug action. For example, there is evidence that DBA/2 mice are more susceptible to convulsant doses of imipenem, a broad spectrum antibiotic of the carbapenem class, than Swiss and NMR1 mice (De Sarro et al., 1995, 1996a). Another example of different susceptibility in DBA/2 mice is obtained with valproate treatment. This antiepileptic drug has a dose-dependent, but modest anticonvulsant action

against seizures induced by the β-carboline, DMCM in DBA/2 mice. However, pretreatment with valproate completely protected Swiss CD-1 mice against DMCM seizures (Chapman et al., 1987). As for caffeine, we found a proconvulsant ED_{50} of 0.04 mg/kg, but higher doses (25–200 mg/kg) are necessary to prolong electroconvulsive seizure durations in rats (Francis and Fochtmann, 1994). Furthermore, aminophylline, another methylxanthine, itself may either lower the convulsive threshold or induce seizures at doses over 200 mg/kg (Chu, 1981; Czuczwar et al., 1987; Cutrufo et al., 1992; De Sarro et al., 1997).

The results of experiments involving combined treatment with adenosine A_1 and A_{2A} receptor-selective agonists and antagonists suggest that, in this genetic model of epilepsy, adenosine A_{2A} receptor agonists have an important role as anticonvulsant agents. This findings is not in agreement with the data reported by Zhang et al. (1994), who studied the anticonvulsant effects of CGS 21680, which does not penetrate sufficiently into the brain (Nikodijevic et al., 1990), and with those reported by Malhotra and Gupta (1997) who observed no anticonvulsant effects following i.p. administration of 5′-(*N*-cyclopropyl)carboxamidoadenosine (CPA). The results of the latter study appeared very difficult to compare with those of our experiments, given the number of differences in the two experimental setups (a) species differences, i.e., rats vs. mice, (b) differences due to epileptic models, and (c) the large dose of CPA in comparison to doses of adenosine A_{2A} receptor-selective agonists used in our study.

In conclusion, these data suggest that, in addition to adenosine A_1, A_{2A} receptors, in the DBA-2 mouse model also appear to be involved in the pathogenesis and propagation of behavioural seizures. More data will make it possible to understand this intriguing aspect better.

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