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Repeated treatment with adenosine A_1 receptor agonist and antagonist modifies the anticonvulsant properties of CPPene

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Abstract

The effects of repeated administration of the selective adenosine A_1 receptor agonist 2-chloro-N^6-cyclopentyladenosine (CCPA), the selective adenosine A_2 receptor agonist 2-hexynyl-5′-*N*-ethylcarboxamidoadenosine (2HE-NECA), the non-selective adenosine A_1/A_2 receptor agonist 5′-*N*-ethylcarboxamidoadenosine (NECA), the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3 dipropylxanthine (DPCPX) and the selective adenosine A_2 receptor antagonist 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-(4,3-e)1,2,4-triazolo(1,5-c)pyrimidine (SCH 58261) on the anticonvulsant activity of 3-(2-carboxypiperazine-4y)propenyl-1-phosphonic acid (CPPene), a selective *N*-methyl-D-aspartate receptor antagonist, were evaluated in audiogenic sensible dilute brown agouti mice DBA/2J (DBA/2). Mice were treated intraperitoneally twice daily for 7 days with CCPA 0.11 mg/kg, 2HE-NECA 0.056 mg/kg, NECA 0.11 mg/kg, DPCPX 0.5 mg/kg and SCH 58261 0.5 mg/kg followed by 2 vehicle injections (the wash-out period of 1 day) and subsequently CPPene was administered intracerebroventricularly. Audiogenic seizures were delivered 30 min after CPPene administration. Repeated treatment with CCPA significantly reduced the anticonvulsant properties of CPPene against audiogenic seizures. A weak and not significant reduction of anticonvulsant effects of CPPene was observed following repeated administration of NECA, whilst the repeated administration of 2HE-NECA did not decrease the antiseizure activity of CPPene. Conversely, repeated administration of DPCPX markedly potentiated the anticonvulsant properties of CPPene, whilst the repeated treatment with SCH 58261 did not increase the anticonvulsant activity of CPPene. The present results indicate that repeated treatment with CPPA, a selective adenosine A_1 receptor agonist, decreases the anticonvulsant properties of CPPene, whilst the repeated administration of DPCPX, a selective adenosine A_1 receptor antagonist, potentiates the anticonvulsant effects of CPPene. The compounds acting as selective agonists or antagonists of adenosine A_2 receptors do not affect the antiseizure activity of CPPene. In conclusion, the repeated interaction of agonists or antagonists with adenosine A_1 receptors seems to induce changes on anticonvulsant activity of CPPene, whereas drugs acting at adenosine A_2 receptors do not.

Keywords: Adenosine analog; Audiogenic seizure; 3-(2-carboxypiperazine-4y)propenyl-1-phosphonic acid (CPPene); Adenosine A_1 receptor agonist; Adenosine A_1 receptor antagonist; Adenosine A_2 receptor agonist; Adenosine A_2 receptor antagonist; (DBA/2 mouse); 2-chloro-N^6-cyclopentyladenosine (CCPA); 8-cyclopentyl-1,3 dipropylxanthine (DPCPX); SCH 58261; 5′-*N*-ethylcarboxamidoadenosine (NECA); 2-hexynyl-5′-*N*-ethylcarboxamidoadenosine (2HE-NECA); Seizure; Epilepsy

1. Introduction

It has been known for some time that adenosine depresses neuronal activity within the central nervous system (CNS) (Phillis and Wu, 1983). Endogenous adenosine possesses a protective role in the CNS (Barraco et al., 1986; Dragunow and Faull, 1988) by inhibiting calcium influx and opening presynaptic potassium channels with a consequent reduction of the release of excitatory amino acids (Ribeiro, 1991). Adenosine A_1 receptor agonists showed remarkable neuroprotective activity when ischaemia and epilepsy occurred (Miller and Hsu, 1992; Rudolphi et al., 1992; De Sarro et al., 1991; De Sarro et al., 1996; Von Lubitz et al., 1993b). Recently, Adami et al. (1995) reported that the selective adenosine A_2 receptor agonist 2-hexynyl-5′-*N*-ethylcarboxamidoadenosine (2HE-NECA) and the non-selective adenosine A_1/A_2 receptor agonist 5′-*N*-ethylcarboxamidoadenosine (NECA) were also

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able to show neuroprotective properties against pentylenetetrazole-induced seizures. Although the protective effects of acute administration of various adenosine agonists in experimental models of epilepsy have been well documented, only a few studies have assessed the neuropharmacological effects of compounds acting as selective adenosine agonists or antagonists following repeated treatment.

In the present study, we reported the effects of a repeated treatment with 2-chloro-*N*-cyclopentyladenosine (CCPA) or 8-cyclopentyl-1,3-dimethylxanthine (DPCPX), acting as selective agonist and antagonist at the adenosine A_1 receptor, and those of NECA a non-selective adenosine A_1/A_2 receptor agonist, 2HE-NECA, a selective adenosine A_2 receptor agonist and 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-(4,3-e)1,2,4-triazolo(1,5-c) pyrimidine (SCH 58261), a selective adenosine A_2 receptor antagonist on the anticonvulsant properties of 3-(2-carboxypiperazine-4)propenyl-1-phosphonic acid) (CPPene) in sensible dilute brown agouti mice DBA/2J (DBA/2), a genetically epileptic rodent model in which seizures were elicited using on auditory stimulation. CPPene, a selective *N*-methyl-D-aspartate (NMDA) receptor antagonist (De Sarro and De Sarro, 1992), was administered intracerebroventricularly (i.c.v.) in order to esclude possible pharmacometabolic and/or pharmacokinetic interferences between CPPene and the compounds acting on adenosine receptors.

2. Materials and methods

2.1. Animals

Male DBA/2 mice (Charles River, Calco, Como, Italy) weighing 7–8 g and 21 days old at the beginning of experiments were used.

2.2. Anticonvulsant activity of CCPene in DBA/2 mice

DBA/2 mice were exposed to auditory stimulation 30 min following i.c.v. administration of vehicle or CPPene (33, 66, 100, 330 and 660 nmol/mouse). For i.c.v. injections, 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 701 N) fitted with a nylon cuff on the needle as previously described (De Sarro et al., 1988). Injections of drugs by this procedure led to a uniform distribution throughout the ventricular system within 10 min (De Sarro et al., 1988). Each mouse was then placed under a hemispheric Perspex dome (diameter 58 cm) and 1 min was allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 1 min or until tonic extension occurred. As previously reported, seizure response (De Sarro et al., 1984) was assessed using the following scale: 0, no response; 1, wild running; 2, clonus; 3, tonus; 4, respiratory arrest. The maximum response was recorded for each animal. Rectal temperature was recorded immediately prior to auditory testing using an Elektrolaboratoriet thermometer type T.E.3. Behavioural changes were observed during the period between drug administration and auditory testing. Latency of the onset of various phases of audiogenic seizures and incidence of each phase of seizures were recorded.

2.3. Repeated treatment with adenosine agonists and antagonists

Six groups each of 50 DBA/2 mice received, respectively, CCPA (0.11 mg/kg), DPCPX (0.5 mg/kg), NECA (0.11 mg/kg), 2HE-NECA (0.056 mg/kg) or SCH 58261 (0.5 mg/kg) twice daily for 7 days intraperitoneally (i.p.). The doses of the compounds acting on adenosine receptors correspond to the ED_{50} values which were able to block audiogenic seizures in DBA/2 mice (De Sarro et al., 1996). For comparison, the control group received repeated treatment with vehicle for the entire lenght of the experiments (twice daily for 7 days).

To exclude the possibility of neurological impairment caused by repeated administration of CCPA, DPCPX, NECA, 2HE-NECA or SCH 58261 separate groups of mice ($n = 10$/group) were also tested at the end of the wash-out period on a rotarod revolving at 5 rpm.

2.4. Receptor binding assay

The brain were removed either immediately upon death or at survival end-point, i.e. 24 h after the injection of CPPene, and frozen on dry ice. Subsequently, they were separated according to the experimental protocol (i.e. either vehicle + CPPene, DPCPX + CPPene, SCH 58261 + CPPene, 2HE-NECA + CPPene, NECA + CPPene or CCPA + CPPene) and to the manner in which they were obtained (i.e. post-mortem or post-sacrificio). Forebrains in each category were then randomly subdivided into various subgroups and homogenized in 10 vols. (v/w) of 0.32% sucrose solution. The homogenate was centrifuged at 1000 $\times g$ for 10 min and the supernatant was removed and recentrifuged at 32 000 $\times g$ for 40 min. The resulting pellet was resuspended in Tris-HCl buffer (pH 7.4) at a concentration of 1.5–2 mg protein/ml. All the above procedures were carried out at 4°C. Protein content was determined using the BCA protein assay reagents (Pierce, Rockford, IL, USA). [^{3}H] DPCPX (Du Pont NEN, Boston, MA, USA) saturation studies were carried out as previously described (Bruns et al., 1987) at 25°C, using the radioligand in the range of 0.04–3 nM. Each incubation tube contained $\approx$ 30–50 μg protein in a total volume of 500 μl Tris-HCl, pH 7.4, with adenosine deaminase (3 IU/ml) present.

Scatchard analysis was used to determine B_{max} and K_d.

2.5. Statistical analysis

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

2.6. Drugs

2-Chloro-N^6-cyclopentyladenosine (CCPA), 8-cyclopentyl-1,3dipropylxanthine (DPCPX) and 5′-*N*-ethylcarboxamidoadenosine (NECA), were purchased from Research Biochemicals (Natick, MA, USA), 2-hexynyl-5′-*N*-ethylcarboxamidoadenosine (2HE-NECA), and 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-(4,3-e)1,2,4-triazolo (1,5-c) pyrimidine (SCH 58261) were kindly supplied by Dr. E. Ongini (Schering-Plough, Milan, Italy). 3-(2-carboxypiperazine-4)propenyl-1-phosphonic acid (CPPene) was kindly supplied by Dr. P.L. Herrling (Sandoz, Berne, Switzerland). The drugs were dissolved in a 5% solution of carboxymethylcellulose and administered i.p. (0.1 ml/10 g body weight), while CPPene was dissolved in phosphate buffer (pH 7.4, 67 mM) and administered i.c.v. in a volume of 5 μl/mouse as previously described (De Sarro et al., 1994, 1995). All adenosine agonists and antagonists were administered twice daily for 7 days i.p. (0.1 ml/10 g body weight). After an 1-day vehicle injection (wash-out period), a dose-response curve of the anticonvulsant activity of CPPene was done.

3. Results

3.1. Effects of repeated treatment with CCPA, DPCPX, NECA, 2 HE-NECA and SCH 58261

Repeated treatment with CCPA, DPCPX, NECA, 2HE-NECA and SCH 58261 alone produced no qualitative changes of spontaneous movements as compared to normal mice and all animals stayed indefinitely on the rotarod.

3.2. Administration of CPPene following acute or repeated vehicle

When CPPene (33, 66, 100, 330 and 660 nmol/mouse i.c.v.) was administered 30 min before sound stimulation, significantly antagonized the clonic and tonic phase of the audiogenic seizures in a dose-dependent manner (Table 1). ED_{50} values against clonic and tonic seizures are reported in Table 2. No significant changes on incidence and latency of onset of audiogenic seizure phase were observed when animals treated repeatedly with vehicle, received CPPene (Table 1).

I.c.v. administration of vehicle + CPPene at 660 nmol/mouse had no effect on either B_{max} or K_d (Table 4). Treatment with CPPene alone or in the vehicle-treated group induced a qualitative reduction of spontaneous movements as compared to normal mice but did not significantly affect the rotarod performance of mice (data not shown).

3.3. Administration of CCPene following repeated DPCPX

Following repeated treatment with DPCPX the injection of CPPene caused a significant ($P < 0.05$) increase of its anticonvulsant activity against audiogenic seizures (Table

Table 1
The effects of i.c.v. administration of CPPene in combination with vehicle, CCPA or DPCPX i.p. in DBA/2 mice

Repeated treatment	Dose (nmol)	% Response				SR mean ± S.E.M.)	*T* (°C,
		WR	Clonus	Tonus	RA		
Phosphate buffer		100	100	100	60	3.6	37.7 ± 0.31
Carboxymethylcellulose + CPPene	33	100	100	100	40	3.4	37.6 ± 0.34
	66	100	100	100	20	3.2	37.4 ± 0.28
	100	100	70	50 [a]	10 [a]	2.3	37.2 ± 0.33
	330	90	40 [b]	30 [b]	10 [a]	1.7	37.0 ± 0.24
	660	20	0 [b]	0 [b]	0 [b]	0.2	36.9 ± 0.26
CCPA + CPPene	33	100	100	100	50	3.5	37.5 ± 0.25
	66	100	100	100	40	3.2	37.2 ± 0.27
	100	100	100	90	30	3.2	37.0 ± 0.26
	330	100	70	60	20	2.5	36.8 ± 0.21 [a]
	660	100	30 [b]	20 [b]	10 [a]	1.6	36.6 ± 0.22 [a]
DPCPX + CPPene	33	100	100	100	30	3.3	37.6 ± 0.28
	66	100	90	80	10 [a]	2.8	37.5 ± 0.22
	100	80	50 [a]	30 [b]	0 [b]	1.7	37.3 ± 0.31
	330	60	10 [b]	10 [b]	0 [b]	0.8	37.1 ± 0.26
	660	0	0 [b]	0 [b]	0 [b]	0	37.0 ± 0.30

Groups of DBA/2 mice ($n = 10$/dose) were injected i.p. with carboxymethylcellulose (vehicle) + CPPene or CCPA + CPPene or DPCPX + CPPene for 7 days and exposed to auditory stimulation 30 min after i.c.v. injection of CCPene or phosphate buffer (67 mM). Incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by [a] $P < 0.05$; [b] $P < 0.01$ using Fisher's exact probability test. WR, wild running; RA, respiratory arrest; SR, mean maximal seizure response (see Section 2 for grading). *T* is rectal temperature measured immediately before auditory stimulation. Significant differences between rectal temperature in drug-treated and control group are denoted by [a] $P < 0.05$ and [b] $P < 0.01$.

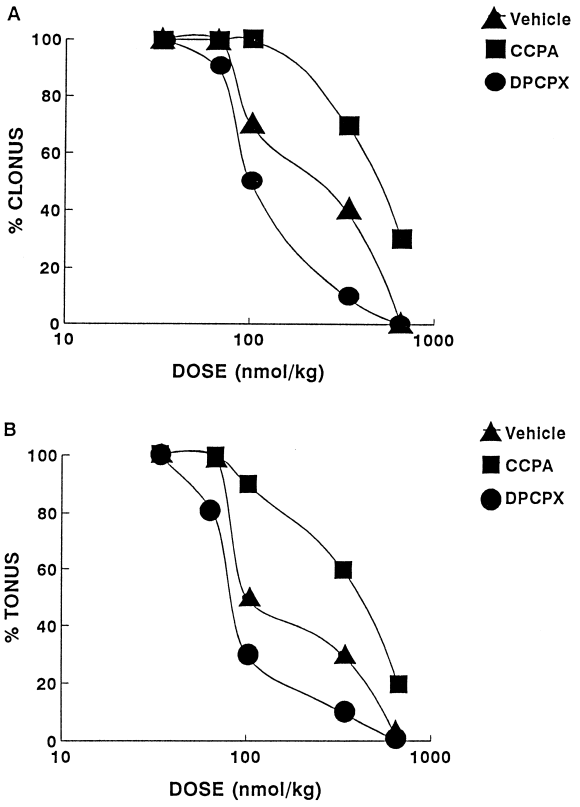

Fig. 1. Dose-response curves observed following CPPene i.c.v. injection in DBA/2 mice pre-treated for 7 days with carboxymethylcellulose, DPCPX and CCPA. A, % clonus; B, % tonus.

1). In particular, following the higher doses of CPPene (100, 330 and 660 nmol i.c.v.) we observed a significant protection against the clonic and tonic phase of the audiogenic seizures (Fig. 1). No significant changes on latency of onset of audiogenic seizures were seen. All animals showed a qualitative reduction of spontaneous movements as compared to normal mice following administration of CPPene. However, the depressed animals were stimulus sensitive, responding to both touch and sharp noises by a rapid translocation to a different location within the cage. Neither ataxia nor any other impairment of gait were seen during such translocation. All mice were able to stay indefinitely on the rotarod. No significant changes on latency of onset of audiogenic seizure phase were observed (data not shown).

Table 2
ED_{50} values (±95% confidence limits) for anticonvulsant effects of CPPene following acute or repeated administration of vehicle and some adenosine agonists and antagonists in DBA/2 mice

Treatment	ED_{50} values (95% confidence limits)	
	Clonus	Tonus
CPPene alone	206 (123–346)	162 (98–269)
Vehicle + CPPene	209 (123–354)	160 (99–259)
CCPA + CPPene	418 (332–671) [a]	353 (214–582) [a]
DPCPX + CPPene	132 (78–222)	105 (69–162)
2HE-NECA + CPPene	226 (119–431)	173 (103–293)
NECA + CPPene	249 (122–509)	189 (118–301)
SCH 58261 + CPPene	209 (120–362)	169 (100–287)

Results are expressed as nmol/mouse i.c.v. and showed the dose value antagonizing clonic or tonic phase of the audiogenic seizures. Significant differences between the ED_{50} values of concurrent vehicle- and drug-treated groups are denoted by [a] < 0.05 using the method of Litchfield and Wilcoxon (1949).

Table 3
The effects of i.c.v. administration of CPPene in combination with vehicle, NECA, 2HE-NECA or SCH 58261 i.p. in DBA/2 mice

Repeated treatment	Dose (nmol)	% Response				SR	*T* (°C, mean ± S.E.M.)
		WR	Clonus	Tonus	RA		
Phosphate buffer		100	100	100	60	3.6	37.7 ± 0.31
Carboxyethylcellulose							
+ CPPene	33	100	100	100	40	3.4	37.6 ± 0.34
	66	100	100	100	20	3.2	37.4 ± 0.28
	100	100	70	50 [a]	10 [a]	2.3	37.2 ± 0.33
	330	90	40 [b]	30 [b]	10 [a]	1.7	37.0 ± 0.24
	660	20 [b]	0	0 [b]	0 [b]	0.2	36.9 ± 0.26
2HE-NECA							
+ CPPene	33	100	100	100	50	3.5	37.5 ± 0.25
	66	100	100	100	20	3.2	37.2 ± 0.27
	100	100	80	60	10 [a]	2.4	37.0 ± 0.26
	330	90	40 [b]	30 [b]	10 [a]	1.7	36.8 ± 0.21 [a]
	660	30 [b]	10 [b]	0 [b]	0 [b]	0.4	36.6 ± 0.22 [a]
NECA							
+ CPPene	33	100	100	100	50	3.5	37.6 ± 0.28
	66	100	100	100	30	3.3	37.5 ± 0.22
	100	100	90	70	20	2.8	37.3 ± 0.31
	330	100	40 [b]	30 [b]	10 [a]	1.8	37.1 ± 0.26
	660	50 [a]	20 [b]	10 [b]	0 [b]	0.8	37.0 ± 0.30
SCH 58261							
+ CPPene	33	100	100	100	40	3.4	37.6 ± 0.34
	66	100	100	100	20	3.2	37.4 ± 0.28
	100	100	70	50 [a]	10 [a]	2.3	37.2 ± 0.33
	330	90	40 [b]	30 [b]	10 [a]	1.7	37.0 ± 0.24
	660	20 [b]	10 [b]	0 [b]	0 [b]	0.4	36.9 ± 0.26

Groups of DBA/2 mice ($n = 10$/dose) were injected i.p. with carboxymethylcellulose (vehicle) + CPPene or NECA + CPPene one or SCH 58261 + CPPene or 2HE-NECA + CPPene for 7 days and exposed to auditory stimulation 30 min after i.c.v. injection of CPPene or phosphate buffer (67 mM). Incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by [a] $P < 0.05$; [b] $P < 0.01$ using Fisher's exact probability test. WR, wild running; RA, respiratory arrest; SR, mean maximal seizure response (see Section 2 for grading). *T* is rectal temperature (mean ± S.E.M.) measured immediately before auditory stimulation. Significant differences between rectal temperature in drug-treated and control group are denoted by [a] $P < 0.05$ and [b] $P < 0.01$.

3.4. Administration of CCPene following repeated CCPA

In this group, neither neurological impairment nor changes on latency of onset of audiogenic seizures differed significantly from those seen in animals injected with vehicle + CPPene. However, while in the vehicle + CPPene (100 nmol i.c.v.) group only 50% animals showed

a tonic phase, in the CCPA + CPPene (100 nmol i.c.v.) group it was present in 90% mice. Furthermore, the clonic phase and mortality increased in animals which received repeated administration of CCPA + CPPene (100 nmol i.c.v.) (Table 1, Fig. 1).

3.5. Administration of CPPene following repeated NECA, 2HE-NECA and SCH 58261

No significant changes in the incidence of audiogenic seizures after CPPene injection were observed in DBA/2 mice that received a repeated treatment with 2HE-NECA and SCH 58261 in comparison to group received a repeated treatment with vehicle (Table 3, Fig. 2). However, a weak and not significant reduction of anticonvulsant effects of CPPene was observed following repeated administration of NECA (Fig. 2). No significant changes on latency of onset of audiogenic seizure phase and no impairment of locomotor performance was observed.

3.6. Receptor density after acute or repeated treatment with vehicle + CPPene or after repeated treatment with CCPA, 2HE-NECA, NECA, SCH 58261 or DPCPX + CPPene

No significant changes in B_{max} or K_d were observed after acute or repeated treatment with vehicle + CPPene.

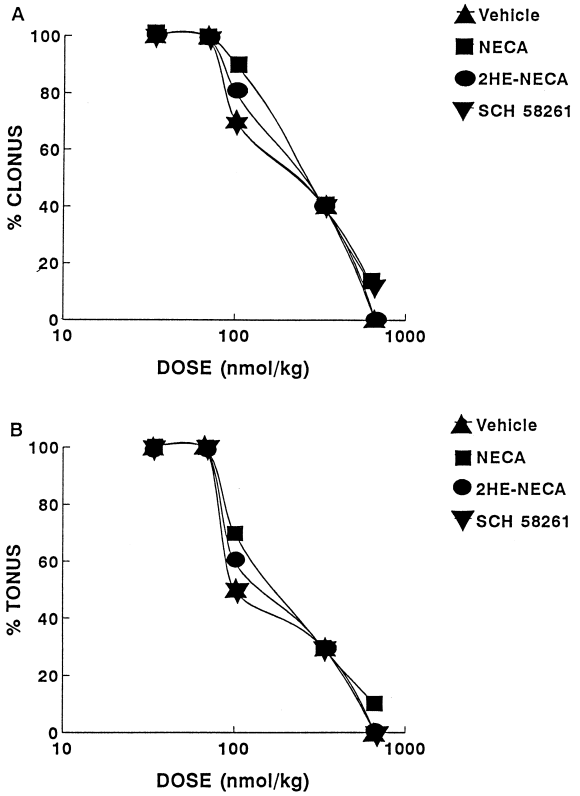

Fig. 2. Dose-response curves obtained following CPPene i.c.v. injection in DBA/2 mice pre-treated for 7 days with carboxymethylcellulose, NECA, 2HE-NECA and SCH 58261. A, % clonus; B, % tonus.

Table 4
Receptor densities (B_{max}) and dissociation constants of adenosine A_1 receptors in animals injected acutely with CPPene (CPPene alone) or repeatedly with vehicle, CCPA, NECA, 2HE-NECA, SCH 58261 or DPCPX + CPPene

Treatment	B_{max}	K_d
CPPene alone	868 ± 38	0.40 ± 0.06
Vehicle + CPPene	846 ± 36	0.44 ± 0.07
CCPA + CPPene	764 ± 32	0.42 ± 0.07
DPCPX + CPPene	986 ± 53	0.40 ± 0.06
NECA + CPPene	785 ± 41	0.43 ± 0.08
2HE-NECA + CPPene	839 ± 35	0.41 ± 0.08
SCH 58261 + CPPene	876 ± 37	0.39 ± 0.06

Values are ± S.E.M. There are no statistically significant differences either in B_{max} or K_d values using Bonferroni's corrected student's t-test. $n = 10$ mice for group. B_{max} data are expressed as fmol/mg tissue or K_d as nM.

Repeated treatment with CCPA, NECA, 2HE-NECA, SCH 58261 or DPCPX did not affect either B_{max} or K_d. Moreover, no significant changes in receptor densities or dissociation constants were seen after repeated treatment with CCPA, NECA, 2HE-NECA, SCH 58261 or DPCPX followed by an administration of CPPene (Table 4). The lenght of postictal survival did not have any influence either, and the values of B_{max} and K_d from animals whose brains were analyzed post-mortem were fully comparable to those examined post-sacrificio (data not shown).

4. Discussion

Several authors have previously described both pro- and anticonvulsant actions of acutely administered adenosine antagonists and agonists in a variety of model of experimental epilepsy (for a review, see Dragunow, 1991). An important result of the present study is that NECA a non-selective adenosine A_1/A_2 receptor agonist, 2HE-NECA a selective adenosine A_2 receptor agonist and CCPA a selective adenosine A_1 receptor agonist showed differences when repeatedly administered on anticonvulsant effects of CPPene as shown by their ED_{50} values.

Although the systemic administration of stable adenosine analogs has been widely proved to induce behavioural changes (Dunwiddie and Worth, 1982; Durcan and Morgan, 1989) and anticonvulsant effects (Dragunow et al., 1985; Berman et al., 1990; Concas et al., 1993), it is unclear to what extent they pass through the blood brain barrier and act directly on central neurons: evidence for (Rosen and Berman, 1985; Durcan and Morgan, 1989) and against (Brodie et al., 1987) has been offered. Unfortunately, our study, though confirming the anticonvulsant effects of peripherally administered adenosine receptor agonists, does not help to resolve this long-standing controversy. However, the relevance of chronic administration of adenosine A_1 receptor agonists has been hitherto un-

known, and only recently Georgiev et al. (1993) reported that the chronically administered non-selective adenosine receptor antagonist caffeine and other non-selective xanthines offer significant protection against NMDA-evoked seizures. Our present observations that the repeated administration of the highly selective adenosine A_1 receptor antagonist DPCPX results in an increase of the anticonvulsant effect of CPPene is consistent with that study. Moreover, the behavioural responses observed are in agreement to those reported following acute administration of the adenosine A_1 receptor agonist CCPA prior to NMDA administration (Von Lubitz et al., 1993b).

Prolonged exposure of rat brain tissues to adenosine analogs results in selective and dose-dependent desensitization of adenosine A_1 receptors (Abbracchio et al., 1993). This observation is supported by in vivo data showing the loss of A_1-mediated anticonvulsant effects after repeated treatment with the adenosine A_1 receptor agonist, N^6-cyclopentyladenosine in NMDA-evoked seizures in mice (Von Lubitz et al., 1994). Conversely, cerebral adenosine A_2 receptor seem to be resistant to agonist-induced desensitization (Abbracchio et al., 1993). In accordance with these observations, our study showed that repeated stimulation of the adenosine A_1 receptor by CCPA leads to tolerance to anticonvulsant activity, whereas both the adenosine A_2 receptor agonist, 2HE-NECA, and the non-selective agonist, NECA, maintain the protective effects of CPPene against sound-induced seizures. In previous studies, it has been demonstrated that, also in the cardiovascular system, repeated administration of CCPA leads to tolerance to both hypotensive and bradycardiac effects, whereas 2HE-NECA and NECA maintain their antihypertensive properties throughout the 21-day experimental period (Casati et al., 1994).

Von Lubitz et al. (1995) have recently suggested that it is highly unlikely that effective concentration of CCPA or DPCPX can be found in the circulation or in the interstitial space of the brain at the end of the 1-day wash-out period. In addition, it is equally unlikely that the observed effects are the result of simple and direct drug-adenosine A_1 receptor interaction. Repeated treatment with the non-selective antagonist caffeine causes significant shifts in the density of radioligand binding sites at acetylcholine, 5-HT, GABA, δ-opioid as well as adenosine A_1 (but not A_2) receptors (Shi et al., 1993; Jacobson et al., 1996). In addition, α-adrenoceptors, dopamine and excitatory amino acid receptors were unalterated (Jacobson et al., 1996). Hence, it is probable that repeated or chronic treatment with ligands of much higher selectivity will also cause complex and long-lasting changes in several receptor systems, many of which may be involved either in generation or spreading of seizures.

A number of prior investigations indicate that the chronic treatment with adenosine A_1 receptor ligands results either in receptor up- or downregulation (Ramkumar et al., 1988; Abbracchio and Cattabeni, 1992) depending whether an antagonist or agonist is used. Although neither Georgiev et al. (1993) nor Von Lubitz et al. (1994) were able to demonstrate such changes, this event cannot be definitively excluded. Nonetheless, it is also possible that the source of the protection against sound-induced seizures rests at the level of coupling of adenosine A_1 receptors to second messenger systems.

Our present results are in agreement with previous data showing that the effects of repeated administered agents acting at the adenosine A_1 receptor are diametrically opposed to those reported following acute administration (Rudolphi et al., 1990; Georgiev et al., 1993; Casati et al., 1994; Adami et al., 1995; Von Lubitz et al., 1993a, 1994). Moreover, such differences may characterize not only the effect of these drugs on excitatory amino acid-evoked seizures, but possibly other processes (e.g. spatial learning and memory) (Normile and Barraco, 1991; Schingnitz et al., 1991; Von Lubitz et al., 1993b). These phenomena were called 'effect inversions' and may depend on drug administration method, frequency, dose and wash-out time before measuring functional changes (Jacobson et al., 1996). Thus, although the 'effect inversions' is not completely clarify, we suggest that the dependence of a therapeutic result on the dosing regimen advocates caution in clinical administration of adenosinergic compounds as it was previously suggested by some authors (see Daval et al., 1991). In addition, this study confirms the well-known anticonvulsant activity of acute administration of adenosine A_1 and A_2 agonists, an action to which tolerance develops when the A_1 agonist is given repeatedly. Adenosine agonists do not appear to offer a potential as anticonvulsant agents because of the variety of peripheral, mainly cardiovascular, effects associated with the central action (Dunwiddie and Worth, 1982; Monopoli et al., 1994a,b). These studies help, however, to clarify the mechanisms governing the different actions of the adenosine A_1 compared with A_2 and perhaps A_3 receptors in the CNS.

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