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Effect of adenosine receptor modulation on pentylenetetrazole-induced seizures in rats

Jatinder Malhotra & [1]Yogendra K. Gupta

Neuropharmacology Laboratory, Department of Pharmacology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India

1 The effects of adenosine, the adenosine analogue, 2-chloroadenosine (2-CADO), the specific adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA) and A_2 receptor agonist 5′-(N-cyclopropyl) carboxamidoadenosine (CPCA), were examined against seizures induced by acute administration of pentylenetetrazole (PTZ), 60 mg kg^{-1}, and PTZ kindled seizures, in rats.

2 Adenosine 1000 mg kg^{-1}, i.p., 5 min pretreatment and CPA 10 mg kg^{-1} i.p., 60 min pretreatment, showed significant protection against acute PTZ-induced seizures while, CPCA up to 10 mg kg^{-1} was ineffective. The adenosine analogue 2-CADO in a dose of 5 mg kg^{-1} was only partially protective and on increasing the dose to 10 mg kg^{-1}, this protection was lost.

3 Theophylline, a non specific adenosine receptor antagonist at 50 mg kg^{-1} and the specific adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), at 1 mg kg^{-1}, if administered before the maximally protective doses of adenosine and CPA, completely reversed the protection afforded by them against PTZ seizures. While, pretreatment with the adenosine A_2 receptor antagonist, 3,7-dimethyl-1-propargylxanthine (DMPX), failed to reverse the protection.

4 Adenosine and the adenosine A_1 receptor agonist in doses that protected against seizures after acute PTZ administration, offered only incomplete protection when tested against PTZ kindled seizures.

5 The effects of adenosine and adenosine receptor agonists on mean arterial pressure, heart rate and rectal temperature were studied, to rule out the possibility of their systemic effects mediating the protection of PTZ seizures. All these agents produced a fall in mean arterial pressure, heart rate and hypothermia in the doses exhibiting an anticonvulsant response. While the effect on blood pressure and heart rate was immediate i.e. seen within 5 min and, maintained throughout the observation period, the development of hypothermia lagged behind the onset of hypotension and bradycardia. However, there was no correlation between haemodynamic and hypothermic response and the anticonvulsant effect.

6 The results indicate that the adenosine mediated anticonvulsant effect is via stimulation of A_1 receptors. Hypotension and hypothermia do not appear to contribute to the protection observed with adenosine and the adenosine A_1 receptor agonists.

Keywords: Adenosine; 2-chloroadenosine (2-CADO); N^6-cyclopentyladenosine (CPA); 5′-(N-cyclopropyl) carboxamidoadenosine (CPCA); pentylenetetrazole (PTZ); PTZ kindled seizures; 8-cyclopentyl-1,3-dipropylxanthine (DPCPX); theophylline; 3,7-dimethyl-1-propargylxanthine (DMPX)

Introduction

Adenosine has been implicated in the spontaneous and abrupt arrest of seizures (Dragunow, 1986). The anticonvulsant action of adenosine and its analogues has been observed in several different studies. In *in vitro* studies, adenosine depresses epileptiform activity in hippocampal slices (Dunwiddie, 1980). While, *in vivo*, in rodents, adenosine and its analogues protect against audiogenic (Maitre *et al*., 1974), chemically-induced (Marangos *et al*., 1990; Petersen, 1991) and kindled seizures (Dragunow & Goddard, 1984). Rapid elevations in brain levels of adenosine have also been documented after experimental seizures (Schultz & Lowenstein, 1978; Winn *et al*., 1980) and post-seizures in epileptic patients (During & Spencer, 1992).

Adenosine stimulates two major receptor subtypes A_1 and A_2, which are linked to a multitude of effectors namely, adenylate cyclase, inositol phosphate, potassium channels, calcium channels and neurotransmitter release (Williams, 1990). Both positive and negative modulation of these effector systems have been documented depending on the receptor subtype activated. Thus, while A_1 receptors inhibit, A_2 stimulate adenylate cyclase activity (Londos *et al*., 1980). Similarly, activation of A_1 receptors inhibits the release of acetylcholine (Ribeiro & Sebastiao, 1986) and glutamate (Dolphin & Archer, 1983) while activation of A_2 receptors has an opposite effect i.e. stimulates ischaemia-evoked release of acetylcholine and glutamate (Burke & Nadler, 1988; Brown *et al*., 1990).

Although recent evidence indicates that the anticonvulsant response of adenosine and its analogues is predominantly mediated via an interaction with adenosine receptors of the A_1 subtype (Franklin *et al*., 1989; Zhang *et al*., 1994), the involvement of adenosine A_2 receptors in the modulation of neuronal excitability has also been suggested (Phillis, 1990). Therefore, we investigated the differential effects of adenosine, the adenosine analogue, 2-chloroadenosine (2-CADO), the adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) and A_2 receptor agonist 5′-(N-cyclopropyl) carboxamidoadenosine (CPCA) in seizures induced by pentylenetetrazole (PTZ), a commonly employed chemoconvulsant, in rats. Their effects on seizures induced by both an acute, convulsant dose of PTZ and chronic PTZ administration (kindled rats) were investigated. Since the systemic administration of adenosine and its analogues caused hypotension and hypothermia, which may potentially modulate the convulsant behaviour, the relationship, if any, between their hypotensive or hypothermic effects and their anticonvulsant effects was also examined.

Methods

Animals

Male Wistar rats weighing 150–200 g were used. The animals were group housed in plastic cages and maintained under

[1] Author for correspondence.

standard laboratory conditions with a natural light-dark cycle. The rats were acclimatized to the environment for a week before the experiments. Each treatment group comprised of 8–10 animals. The animals were used only once in the study.

Experimental procedures

Pentylenetetrazole seizures PTZ (Sigma, U.S.A.) was dissolved in normal saline and different doses i.e. 80, 70, 60 and 50 mg kg^{-1} were tested. The dose of 60 mg kg^{-1} consistently produced generalized clonic seizures with minimal mortality (11.1%) and was used for acute challenge throughout the study. The animals were observed for 30 min after PTZ challenge. The % incidence and latencies of (a) myoclonic jerks and (b) generalized clonic seizures with falling were noted. The latencies were numerically transformed to a seizure score and plotted as fold change in latency as described previously (Dunwiddie & Worth, 1982). Briefly, seizure score (S) was calculated from the following formula,

$$S = 1 - (\text{control latency}/\text{drug seizure latency})$$

In the case of control animals, $S = 0$, whereas, for animals that did not experience seizures, i.e. have seizure latencies of infinity, $S = 1$. This numerical transformation enables inclusion of all animals in the statistical analysis, irrespective of whether they have seizures or not. The mortality in the 24 h following PTZ, in the different pretreatment groups was also recorded.

Pentylenetetrazole kindling Animals were injected with a subconvulsant dose of PTZ i.e. 30 mg kg^{-1}, i.p., on alternate days, three times a week. The rats were observed for 30 min after the subconvulsant PTZ and seizure activity scored using a scoring system from 0 to 5 (Table 1). Animals showing five stage 5 seizures not necessarily consecutive were considered to be kindled after which, the PTZ treatment was stopped. To ascertain whether the increased sensitivity to PTZ is persistent, the rats were challenged with subconvulsant PTZ (30 mg kg^{-1}) on the 3rd and 10th day after PTZ treatment had ended. Only rats which had a stage 5 seizure on both the days were used for experiments with different drug treatments.

Effects on blood pressure and heart rate Rats were anaesthetized with urethane 140 mg $100g^{-1}$. The jugular vein, trachea and carotid artery were exposed and cannulated with polyethylene cannulae. The carotid cannula was connected to a pressure transducer for continuous monitoring of blood pressure. The pressure transducer was kept at the level of the animal and connected to the input of S72-25, transducer coupler type A (strain gage bridge), of Videograph II (Coulbourn Instruments, U.S.A.). The output of this was connected to the L25-12, 12 bit analogue input, which can receive input from channels 1 through to 8. The signals were concurrently converted in an interface and fed into the computer. The data were stored on hard disk for off-line analysis. Data acquistion was performed by use of CODAS software of Coulbourn Instruments Inc., U.S.A.

Effect on body temperature Rats were placed in perspex restrainers at room temperature (25–28°C). The rectal temperature was continuously recorded for two hours following administration of adenosine and its analogues, by a rectal probe connected to a six channel telethermometer.

Table 1 Scoring system for PTZ kindled seizures

Stage 1	Hyperactivity, restlessness, vibrissae twitching
Stage 2	Head nodding, head clonus, myoclonic jerks
Stage 3	Unilateral or bilateral limb clonus
Stage 4	Forelimb clonic seizures
Stage 5	Generalized clonic seizures with falling

Drugs and treatment schedules

All drugs were prepared freshly and administered intraperitoneally, in a volume not exceeding 1 ml $100g^{-1}$, by a 25 gauge hypodermic needle. Adenosine (Sigma, U.S.A.) was suspended in 8% Tween 20 and administered in doses of 500 and 1000 mg kg^{-1}, 5, 15 and 60 min before PTZ challenge. N^6-cyclopentyladenosine (CPA; Sigma, U.S.A.), an adenosine A_1 receptor agonist was dissolved in 8% ethanol and injected 30 and 60 min before PTZ in doses of 1, 2, 5 and 10 mg kg^{-1}. The adenosine receptor agonist 2-chloroadenosine (2-CADO; RBI, U.S.A.) was dissolved in normal saline and given in doses of 5 and 10 mg kg^{-1}, 30 min before PTZ in another group of rats. 5′-(N-cyclopropyl)carboxamidoadenosine (CPCA; Sigma, U.S.A.), an adenosine A_2 receptor agonist, was suspended in 8% Tween 20 and administered in doses of 1 and 10 mg kg^{-1}, 60 min before PTZ. The adenosine receptor antagonists used were the nonspecific adenosine receptor antagonist, theophylline (Sun Pharma, India) the specific adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; RBI, U.S.A.) and the specific adenosine A_2 receptor antagonist, 3,7-dimethyl-1-propargylxanthine (DMPX; RBI, U.S.A.). These were dissolved in warm saline, DMSO and distilled water, respectively. Theophylline was given in doses of 10, 20 and 50 mg kg^{-1}, DPCPX in doses of 0.1, 1 and 5 mg kg^{-1} and DMPX 1 mg kg^{-1}, before pretreatment with the maximally protective dose of adenosine (1000 mg kg^{-1}) and CPA (10 mg kg^{-1}), which was followed by convulsant (PTZ) challenge. The pretreatment times for theophylline were 5 and 40 min before CPA and adenosine, respectively, and for DPCPX and DMPX, 5 min and 15 min, respectively. Control experiments were performed with the vehicles used for the different drugs.

Data analysis

The results were analysed statistically by use of a microsoft computer programme microstat, copyright Ecosoft Inc., U.S.A. Fisher's exact test was applied for incidence of seizures.

Results

In control experiments PTZ, 60 mg kg^{-1}, challenge produced myoclonic jerks and generalized clonic seizures in 100% of the animals. Pretreatment with different vehicles used (distilled water, saline, 8% Tween 20, DMSO and 8% ethanol) had no effect on either incidence or latency of PTZ seizures as compared to PTZ alone.

Figure 1 shows the change in % incidence of myoclonic jerks and clonic seizures during PTZ seizures following pretreatment with adenosine, CPA, 2-CADO and CPCA. The corresponding change in latencies are shown in Figure 2. Of the two doses of adenosine tested (500 and 1000 mg kg^{-1}, i.p., at different pretreatment time schedules), 1000 mg kg^{-1}, 5 min pretreatment was maximally effective. Although a nearly 2 fold increase in latency was seen with both the 500 mg kg^{-1}, 5 min and 1000 mg kg^{-1}, 15 min pretreatment schedule.

When the different doses (1, 2, 5 and 10 mg kg^{-1}) of the specific A_1 agonist, CPA, were administered 30 min before PTZ, there was no significant reduction in seizure activity as evident from the 100% seizure incidence and moderate but inconsistent increase in latencies (data not shown). The 60 min pretreatment resulted in greater protection and the effect was dose-dependent. The other adenosine agonist, 2-CADO, 5 mg kg^{-1} 30 min pretreatment, also offered significant protection. However, when the dose was increased to 10 mg kg^{-1}, this protection was not observed. With CPCA pretreatment, there was no significant change in either incidence or latency of seizures. In all the other groups mentioned above, except CPCA, there was no significant mortality in the 24 h post-seizures. With CPCA pretreatment there was 60% mortality.

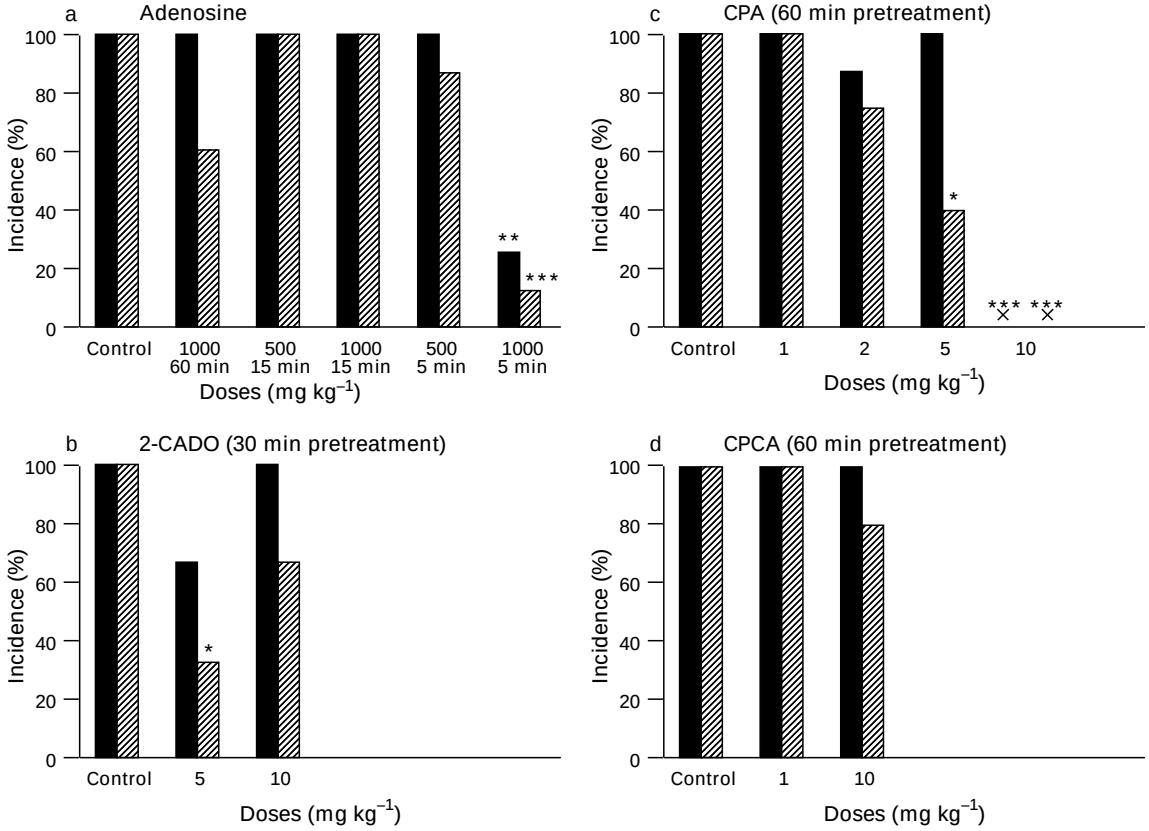

Figure 1 Effect of pretreatment with (a) adenosine, (b) 2-chloroadenosine (2-CADO), (c) N^6-cyclopentyladenosine (CPA) and (d) 5′-N-cyclopropyl)carboxamidoadenosine (CPCA) on % incidence of myoclonic jerks (solid columns) and generalized clonic seizures (hatched columns) in pentylenetetrazole-induced (acute administration) seizures in rats. $^*P<0.05$, $^{**}P<0.005$, $^{***}P<0.001$.

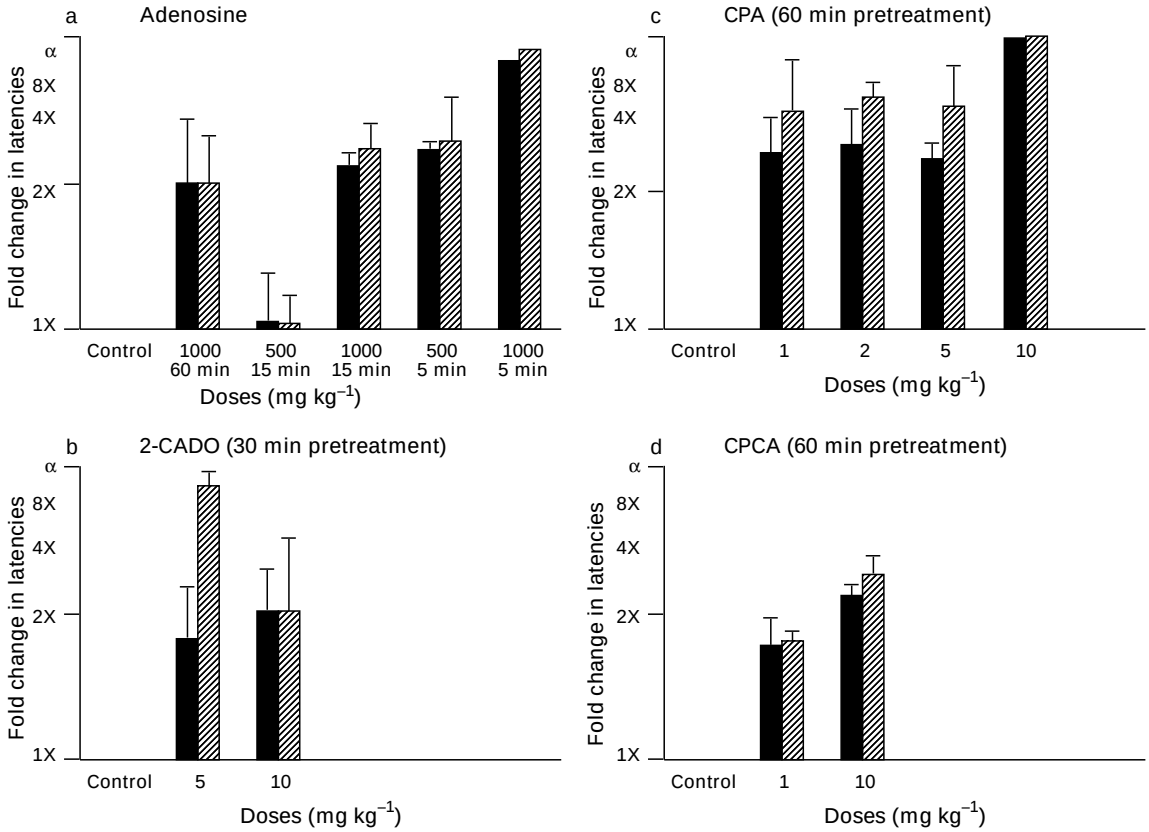

Figure 2 Effect of pretreatment with (a) adenosine, (b) 2-chloroadenosine (2-CADO), (c) N^6-cyclopentyladenosine (CPA) and (d) 5′(N-cyclopropyl) carboxamidoadenosine (CPCA) on latencies of myoclonic jerks (solid columns) and generalized clonic seizures (hatched columns) in pentylenetetrazole-induced (acute administration) seizures in rats. $^*P<0.05$, $^{**}P<0.005$, $^{***}P<0.001$.

Theophylline pretreatment dose-dependently reversed the protection of adenosine as well as of CPA. A complete reversal was seen with 50 mg kg^{-1} and the mortality during the observation period (30 min) was 83.3% and 100% in adenosine- and CPA-treated groups, respectively. The adenosine A_1 receptor antagonist, DPCPX 1 mg kg^{-1} pretreatment, also completely reversed the protection afforded by adenosine and CPA. However, in this group there was no mortality. On the other hand, DMPX, the adenosine A_2 receptor antagonist, 1 mg kg^{-1}, did not significantly reverse the protection of adenosine (Figure 3) or CPA (Figure 4). Neither theophylline, DPCPX and DMPX in the doses used, *per se*, caused convulsions nor did they significantly alter the incidence or latency of PTZ seizures (acute administration). Increasing the dose of DPCPX and DMPX to 5 mg kg^{-1} also did not have a deleterious effect on seizures (data not shown).

The chemically kindled animals were administered the maximally protective doses of adenosine and its agonists, as determined in experiments with acute challenge of PTZ. Interestingly, in none of these groups was complete protection of seizure activity seen. Even though with CPA, 10 mg kg^{-1}, only 28.6% animals had stage 5 seizures, all the rats experienced stage 4 seizures (Table 2).

Effect on blood pressure and heart rate

There was a significant fall in both blood pressure and heart rate after adenosine, 2-CADO, CPA and CPCA. With all the agents, the maximum fall was seen between 5 to 15 min after drug administration and was maintained up to 2 h (observation period). Only in the adenosine-treated groups did the blood pressure and heart rate tend to recover 2 h post treat-

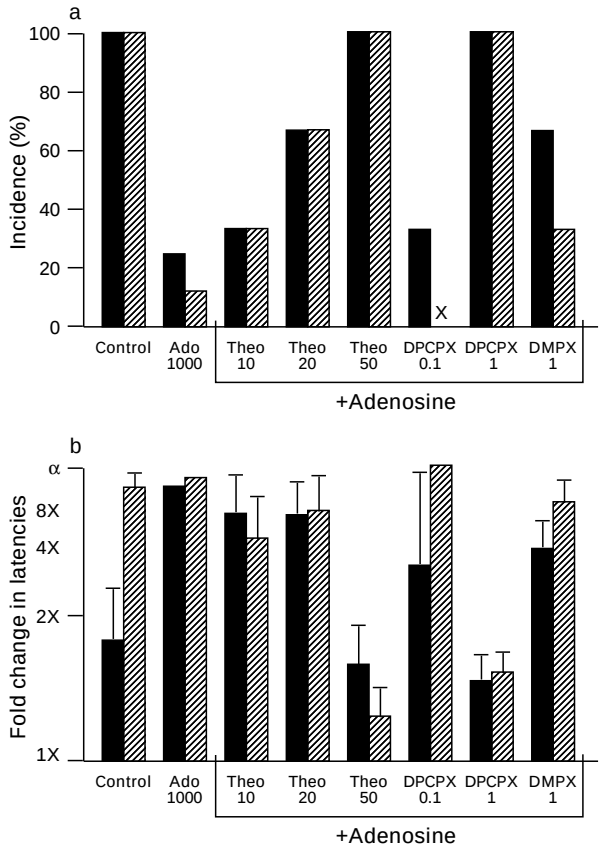

Figure 3 Effect of pretreatment with different doses (shown as mg kg^{-1}) of theophylline (Theo), A_1 receptor antagonist, DPCPX, and A_2 receptor antagonist, DMPX, before adenosine (Ado) 1000 mg kg^{-1} on (a) % incidence and (b) latencies of myoclonic jerks (solid columns) and generalized clonic seizures (hatched columns) in pentylenetetrazole-induced (acute administration) seizures in rats. $*P<0.05$, $**P<0.005$, $***P<0.001$.

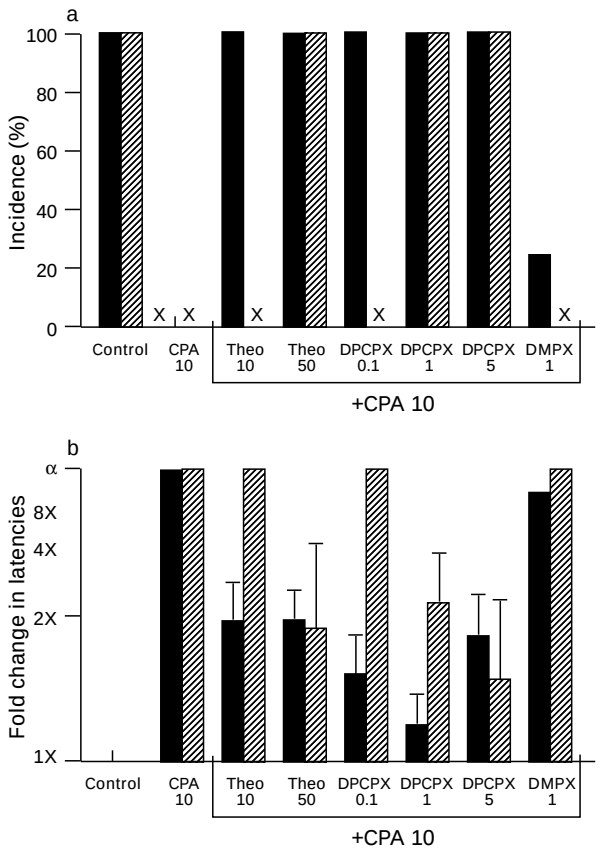

Figure 4 Effect of pretreatment with different doses (shown as mg kg^{-1}) of theophylline (Theo), A_1 receptor antagonist, DPCPX and A_2 receptor antagonist, DMPX before CPA 10 mg kg^{-1} on (a) % incidence and (b) latencies of myoclonic jerks (solid columns) and generalized clonic seizures (hatched columns) in pentylenetetrazole-induced (acute administration) seizures in rats. $*P<0.05$, $**P<0.005$, $***P<0.001$.

Table 2 Effect of adenosine, 2-chloroadenosine (2-CADO), N^6-cyclopentyladenosine (CPA) and 5′(N-cyclopropyl) carboxamidoadenosine (CPCA) on PTZ kindled seizures

S. No.	*Drug*	*Dose* (mg kg^{-1})	*Pretreatment time* (min)	*Mean score*	*Seizure score**
1	Control	–	–	5.00±0.00	0
2	Adenosine	1000	5	4.12±0.54	0.65±0.14
3	2-CADO	5	30	5.00±0.00	−0.22±0.48
4	CPA	10	60	4.20±0.43	0.45±0.30
5	CPCA	10	60	5.00±0.00	−0.19±0.30

Each group consisted of 8–10 animals. Values are mean±s.e.mean. *Seizure score is derived from latencies of generalized clonic seizures as described under PTZ-induced seizures.

ment. The fall in mean arterial pressure and heart rate was maximum with CPA. In the case of CPCA (10 mg kg^{-1}), initially the blood pressure decreased as with the other drugs and this was followed by a slight recovery at about 30 min after which time it fell again. The fall in blood pressure corresponded to the decrease in heart rate (Figures 5 and 6).

Effect on core temperature

Adenosine 1000 mg kg^{-1} brought about the greatest fall in temperature while CPCA (10 mg kg^{-1}) caused the least reduction. The time course of the reduction in temperature was nearly the same with all the drugs tested. The fall in temperature lagged behind their hypotensive and bradycardic effects (Figure 7).

Discussion

In the present study adenosine (1000 mg kg^{-1}), and CPA (10 mg kg^{-1}) significantly protected, while CPCA did not show any significant protection, against PTZ-induced seizures. The protective effects of adenosine and CPA could be reversed by both theophylline and DPCPX, but not by DMPX. Theophylline has been shown to possess antagonist activity at adenosine receptors (Schwabe *et al*., 1985) and recently it has been suggested that the convulsant potency of xanthines is related to their antagonist activity at adenosine A_1 receptors (Moraidis & Bingmann, 1994). DPCPX is a highly selective adenosine A_1 receptor antagonist (Bruns *et al*., 1987) and CPA is also highly potent and selective adenosine A_1 receptor agonist (Bruns *et al*., 1986), in addition adenosine has a greater affinity for the A_1 receptor (Olsson & Pearson, 1990). Therefore, these findings point towards a predominantly A_1 adenosine receptor involvement in mediating the anticonvulsant action against PTZ seizures. The protective effect of adenosine A_1 receptor stimulation against chemically-induced seizures has also been observed by others (Von Lubitz *et al*., 1993; Zhang *et al*., 1994). However, the experimental animal models used in their studies were different i.e. NMDA seizures in mice and bicuculline-methiodide-induced seizures, respectively.

The high mortality seen in theophylline pretreated animals is unlikely to be due to adenosine A_1 receptor antagonism, since the A_1 selective antagonist, DPCPX, although reversing the seizure protection, did not cause death of the animals. The convulsant action of theophylline has classically been related to antagonism at the adenosine A_1 receptor site (Cutrufo *et al*., 1992; Moraidis & Bingmann, 1994). However, data indicating an alternative mechanism, i.e. other than adenosine receptor antagonism, have recently been found (Hornfeldt & Larson, 1994; Malhotra *et al*., 1996).

The inability of the adenosine A_2 receptor antagonist, DMPX to reverse significantly the seizure protection of adenosine and CPA rules out the possibility of A_2 receptor involvement in mediating the anticonvulsant response. Interestingly, another adenosine analogue, 2-CADO was only partially effective in doses of 5 mg kg^{-1} and even this protection was not seen when the dose was increased to 10 mg kg^{-1}. Although 2-CADO has a greater affinity for A_1 receptors, the selectivity is much less than that of CPA. It is probable that at the higher doses 2-CADO loses its selectivity and activates A_2 receptors as well. The effects resulting from activation of A_2 receptors have been shown, *in vitro* to prevail over the effects mediated by A_1 receptors. (Ameri & Jurna, 1991).

However, the exact mechanism of the anticonvulsant effect of adenosine and adenosine A_1 receptor stimulation is unclear given the multitude of effector systems linked to the A_1 adenosine receptor. Phillis & Wu (1983) suggested a distinct possibility of peripheral effects of adenosine and adenosine analogues confounding the interpretation of behavioural and electrophysiological experiments in rats. The two peripheral

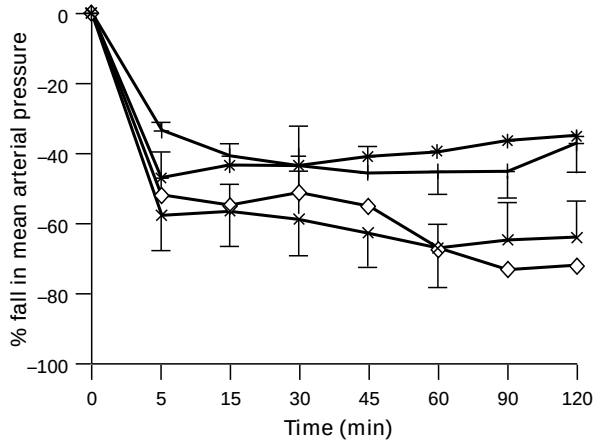

Figure 5 Effect of pretreatment with adenosine (+), 2-chloroadenosine (*), N^6-cyclopentyladenosine (**X**) and 5′-(N-cyclopropyl) carboxamidoadenosine (◇) on mean arterial pressure in rats. Data shown are means and vertical lines indicate s.e.mean.

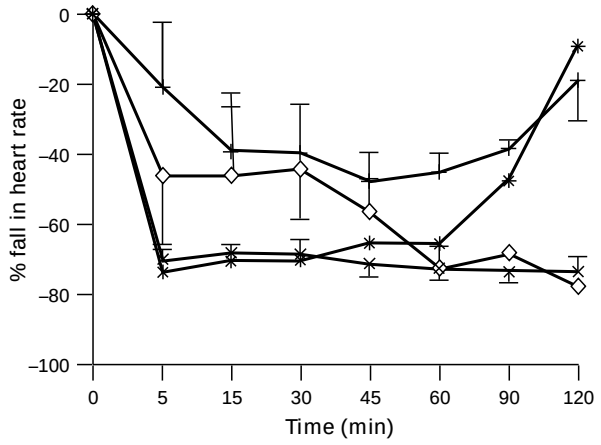

Figure 6 Effect of pretreatment with adenosine (+), 2-chloroadenosine (*), N^6-cyclopentyladenosine (**X**) and 5′-(N-cyclopropyl) carboxamidoadenosine (◇) on heart rate in rats. Values are mean and vertical lines show s.e.mean.

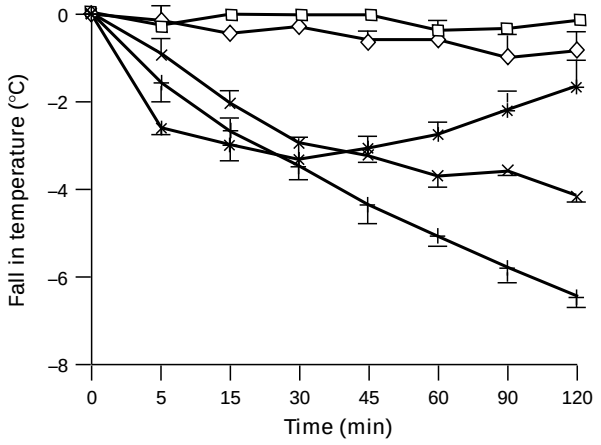

Figure 7 Effect of pretreatment with adenosine (+), 2-chloroadenosine (*), N^6-cyclopentyladenosine (**X**) and 5′-(N-cyclopropyl) carboxamidoadenosine (◇) on rectal temperature in rats (□) Control temperature. Values are means and vertical lines show s.e.mean.

effects of importance following systemic administration of adenosine and adenosine analogues are hypotension and hypothermia. In the present study these factors were examined.

A significant fall in blood pressure, heart rate and body temperature was observed with adenosine, 2-CADO, CPA and CPCA, in doses utilized in experiments with PTZ. Adenosine A_1 receptors have been associated predominantly with a negative inotropic action while A_2 receptor activation causes vasodilatation and consequently hypotension (Jonzon *et al*., 1986). The profound and prolonged fall in blood pressure induced by the selective A_1 agonist CPA could be due to the negative inotropic effect of A_1 adenosine receptor stimulation coupled with the vasodilator effect. Adenosine A_1 receptors are known to mediate a negative inotropic action (Sollevi *et al*., 1984) and, recently, it has also been shown that the vasodilatation of CPA is in part mediated through activation of potassium channels (Merkel *et al*., 1992). A concentration-dependent response of adenosine agonists on renal vasculature and renin secretory responses has also been observed (Pelleg & Porter, 1990). If the anticonvulsant effect of adenosine and A_1 receptor agonists is related to their hypotensive effect, then the adenosine A_2 receptor agonist CPCA which caused a severe and prolonged hypotension should also have exerted a similar anticonvulsant action. However, CPCA had no effect. Therefore, the relationship between hypotensive and anticonvulsant action of adenosine and adenosine receptor agonists seems unlikely. This is further evident from the time course of haemodynamic changes and the anticonvulsant effect. CPA was not effective after 30 min pretreatment whereas it was 100% protective when given 60 min before PTZ challenge. However, in haemodynamic experiments there was no significant difference in blood pressure at 30 min and 60 min following CPA.

A nearly similar time course of the hypothermic effect was observed with adenosine and the A_1 receptor agonists. The hypothermia induced by adenosine, 1000 mg kg^{-1}, was more than that induced by other agonists i.e. CPA, 2-CADO and CPCA. It has previously been shown that the hypothermic effect is mediated by receptors of the A_2 subtype and is secondary to the vasodilator effect, though, central compensatory mechanisms may also contribute (Jonzon *et al*., 1986). In the present study the development of hypothermia also lagged behind the hypotensive response. Apart from this, there is no relationship between the time course of fall in temperature and the anticonvulsant effect. Adenosine 1000 mg kg^{-1} showed its greatest protection at 5 min while the same dose was less effective when given 15 min and 60 min before PTZ challenge, though there was a progressively increasing fall in temperature with increase in time lapse. That hypothermia does not contribute to the anticonvulsant effect of CPA in N-methyl-D-aspartate (NMDA)-induced seizures has been demonstrated by Von Lubitz *et al*. (1993). In their experiments in mice under controlled ambient temperature, there was no significant body temperature difference between animals injected with NMDA alone or CPA with NMDA, but the seizure latency and average time to death were adversely affected in animals injected with NMDA alone, while in the NMDA/CPA group only the seizure latency was significantly reduced.

To date studies on the effects of adenosine and adenosine analogues in PTZ kindling are scarce. In this study adenosine and adenosine analogues only provided weak protection in PTZ kindled seizures as compared to that against seizures induced by an acute convulsant dose of PTZ. This difference can perhaps be accounted for by the dissimilar mechanisms of chronic epileptogenesis (development of kindling induced seizure susceptibility) and those of the acute convulsive reaction to the epileptogen (Kryzhanovskii *et al*., 1990). It is conceivable that chronic treatment with PTZ may bring about certain changes in the neurotransmitter milleu as well as their receptors and effectors. Angelatou *et al*. (1990) have shown that adenosine A_1 receptor density increases after PTZ seizures. Furthermore, *in vitro*, in crude synaptosomal membranes from cortex and hippocampus of mice, an increase in sensitivity to adenosine 48 h after PTZ convulsions has also been observed (Psarropoulou *et al*., 1994). However, results from these studies should not be compared with those from our experiments given the number of variables in the two experimental setups, e.g. (a) species difference i.e. mice vs. rats, (b) the duration or chronicity of the PTZ treatment (3 times/week $\times$ 10 weeks) and, (c) the difference due to *in vitro* and *in vivo* experiments. However, in electrically kindled seizures protection has been observed on administration of adenosine agonists (Dragunow *et al*., 1985). The exact mechanism of PTZ kindled seizures is unknown although an interaction with the γ-aminobutyric acidergic system is suspected. It has also been suggested that differences exist in the neuronal mechanism of PTZ kindling and amygdaloid kindling (Nagai *et al*., 1993). The difference in mechanisms can perhaps account for the discrepancy in our results.

The high mortality observed in the twenty four hours after treatment with the adenosine A_2 receptor agonist CPCA, is difficult to explain. That the A_2 receptors are proconvulsant can be ruled out, as the adenosine A_2 receptor antagonist DMPX *per se*, even up to doses of 5 mg kg^{-1}, did not show any anticonvulsant effect against PTZ-induced seizures. The observed mortality is perhaps due to prolonged hypotension and cardiovascular collapse consequent to A_2 receptor stimulation. The adenosine A_1 receptor agonist CPA, exhibited comparable haemodynamic changes but did not cause mortality, thus raising the possibility of involvement of some additional contributing mechanism(s). Since A_2 agonists have also been demonstrated to increase significantly the ischaemia-evoked release of both aspartate and glutamate (O'Regan *et al*., 1992), it can be conjectured that excitotoxicity, secondary to a profound and prolonged fall in blood pressure, may be responsible for the high mortality. Even though both adenosine and CPA also produced a comparable hypotensive response, at the same time, due to their potent vasodilator effect on cerebral arteries, they improved cerebral blood flow (Van Wylen *et al*., 1991). It is possible that the improved neuronal blood supply coupled with inhibition of release of excitatory neurotransmitters confered an advantage, in terms of survival, in these groups.

In conclusion, our study further substantiates the role of adenosine A_1 receptors in mediating an anticonvulsant effect against PTZ-induced seizures and that hypotension and hypothermia are unlikely to contribute to this action. The results also indicate that adenosine A_2 receptors do not have a role as proconvulsants.

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