



attenuate them. In order to ascertain whether blockade of adenosine A<sub>1</sub> receptor, is involved in the convulsant action of theophylline, the effect of adenosine and specific adenosine A<sub>1</sub> receptor agonist, N<sup>6</sup>-cyclopentyladenosine (CPA) was studied in theophylline-induced seizures in rats. The effect of the established antiepileptic drugs eg. diazepam, sodium valproate, phenobarbitone and carbamazepine, was also evaluated for the purpose of comparison.

## METHODS

Male 'Wistar' rats, weighing 150–200 g were used. The animals were group housed in plastic cages (not more than four animals per cage) and maintained under standard laboratory conditions with a natural light-dark cycle and controlled temperature (20–25°C) and humidity. The rats were acclimatized to the environment for at least a week prior to experimentation. Animals were allowed free access to food and water till, just before the drug treatment. Each treatment group comprised of 8–12 animals. The animals were used only once in the study.

*Theophylline-induced seizures:* Theophylline (courtesy Sun Pharma, India) was dissolved in warm saline and administered intraperitoneally. To establish the dose of theophylline that produces convulsions in 100% animals, it was administered in graded doses i.e., 200, 250, 275 and 300 mg/kg. The dose of 300 mg/kg, i.p., produced seizures in 100% animals and was used for experiments with the different drug treatments.

*Observational parameters:* The animals were observed for one hour following convulsant dose of theophylline and, the percent incidence of clonic seizures and tonic hind limb extension as well as their latencies, were noted. The recording was done manually and supplemented with a seizure recording assembly previously described by us (13). The mortality during the 1 hour observation period and also, the mortality at 24 hours after theophylline challenge was recorded.

*Drugs and treatment schedules:* All drugs were prepared fresh and administered intraperitoneally, in a volume not exceeding 1 ml/100 g, using a 25 gauge hypodermic needle. Control experiments were performed with the vehicles used for the different drugs i.e., 8% tween 20, 8% ethanol, 3% tween 80, saline and distilled water. Doses selected and pretreatment times were decided on the basis of earlier experiments (12, 14).

*Experiments with adenosine and specific adenosine A<sub>1</sub> receptor agonist, N<sup>6</sup>-cyclopentyladenosine (CPA):* Adenosine (Sigma, St. Louis, MO, U.S.A) was suspended in 8% tween 20 and administered in doses of 100, 500 and 1000 mg/kg, i.p., 5 min before theophylline challenge. N<sup>6</sup>-cyclopentyladenosine (CPA Sigma, St. Louis, MO, U.S.A.), an adenosine A<sub>1</sub> receptor agonist was dissolved in 8% ethanol and injected in doses of 1, 5 and 10 mg/kg, i.p., 60. min before convulsant dose of theophylline.

*Experiments with established antiepileptic drugs - a) diazepam, b) sodium valproate, c) phenobarbitone, and d) carbamazepine:* Diazepam (courtesy Intas Laboratories Ltd.,

India) was suspended in 3% tween 80 and administered in a dose of 4 mg/kg, 60 min before theophylline challenge. Sodium valproate (courtesy Reckitt and Colman Ltd., India) was dissolved in distilled water and administered in a dose of 300 mg/kg, 15 min before theophylline. Phenobarbitone (courtesy Intas Laboratories Ltd., India) was dissolved in distilled water by adding a drop of 0.1 N sodium hydroxide. It was injected 60 min before convulsant challenge, in a dose of 50 mg/kg. Carbamazepine (courtesy Intas Laboratories Ltd., India) was dissolved in 40% propyleneglycol, 10% ethanol and 50% water. It was given 30 min before, in a dose of 20 mg/kg.

#### Data analysis

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

## RESULTS

Theophylline 200 mg/kg, i.p. did not produce seizures in any rat. The doses of

250 and 275 mg/kg caused generalized clonic seizures in 83.3% animals in both the groups while the incidence of tonic hind limb extension was 33.3% and 50% respectively. With theophylline 300 mg/kg, 100% animals experienced both generalized clonic seizures and hind limb tonic extension. The mortality during the one hour observation period was 100 percent. Typically, the seizure activity after theophylline 300 mg/kg, comprised of repeated generalized clonic seizures followed by hind limb tonic extension and death. The mean latency to first clonic seizure was  $8.94 \pm 1.31$  min while that of tonic hind limb extension was  $11.44 \pm 1.9$  min.

None of the vehicles used i.e. saline, 8% tween 20, 3% tween 80 or 8% ethanol caused any significant change in any of the seizure components studied.

#### *Effect of adenosine and specific adenosine A<sub>1</sub> receptor agonist N<sup>6</sup>-cyclopentyladenosine (CPA) on theophylline-induced seizures :*

When theophylline challenge was given 5 min after adenosine, at the higher doses i.e. 500 and 1000 mg/kg, there was no

TABLE I : Effect of pretreatment with adenosine (ADO) and adenosine A<sub>1</sub> receptor agonist, N<sup>6</sup>-cyclopentyladenosine (CPA) on latencies in seizures induced by 100% convulsant dose of theophylline (300 mg/kg). Values are mean  $\pm$  S.E.M.

S.No.	Drug & Dose (mg/kg)	Pretreatment time (min)	n	Latencies (min)	
				Clonic seizures	Hind limb extension
1.	Control	-	12	$8.94 \pm 1.31$	$11.44 \pm 1.90$
2.	ADO 100	5	10	$15.68 \pm 0.98$	$28.48 \pm 0.00^*$
3.	ADO 500	5	10	$25.65 \pm 5.39$	$35.21 \pm 8.84$
4.	ADO 1000	5	10	$7.06 \pm 0.92$	$8.94 \pm 0.99$
5.	CPA 1	60	10	$13.10 \pm 1.84$	$18.49 \pm 2.14$
6.	CPA 5	60	10	$28.47 \pm 6.69$	$11.46 \pm 2.05$
7.	CPA 10	60	10	$11.25 \pm 1.32$	$12.85 \pm 2.29$

\*No. of animals showing hind limb toxic extension insufficient to calculate S.E.M.

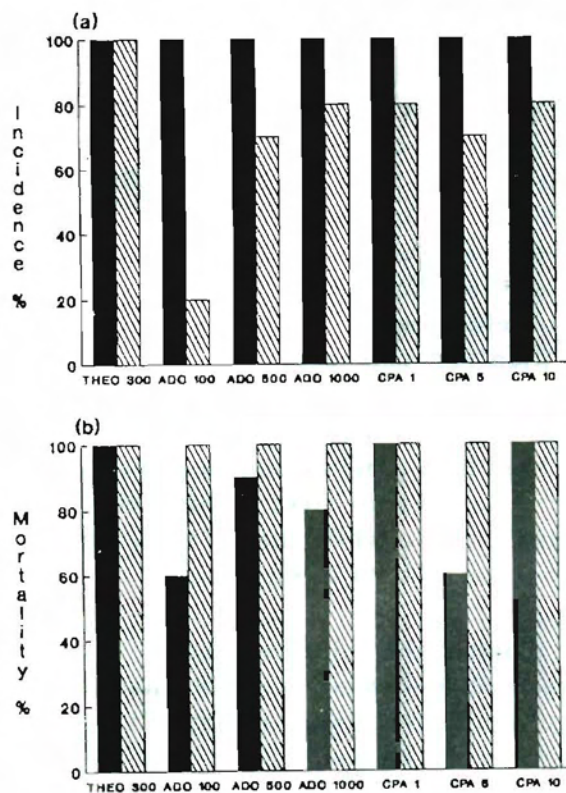


Fig. 1 : Effect of adenosine (ADO) and specific adenosine A<sub>1</sub> receptor agonist, N<sup>6</sup>-cyclohexyl adenosine (CPA) in theophylline-induced seizures. (a) Effect on percent incidence of generalized clonic seizures (solid columns), and tonic hind limb extension (hatched columns), (b) Effect on percent mortality during one hour (solid columns) and in twenty four hours (hatched columns).

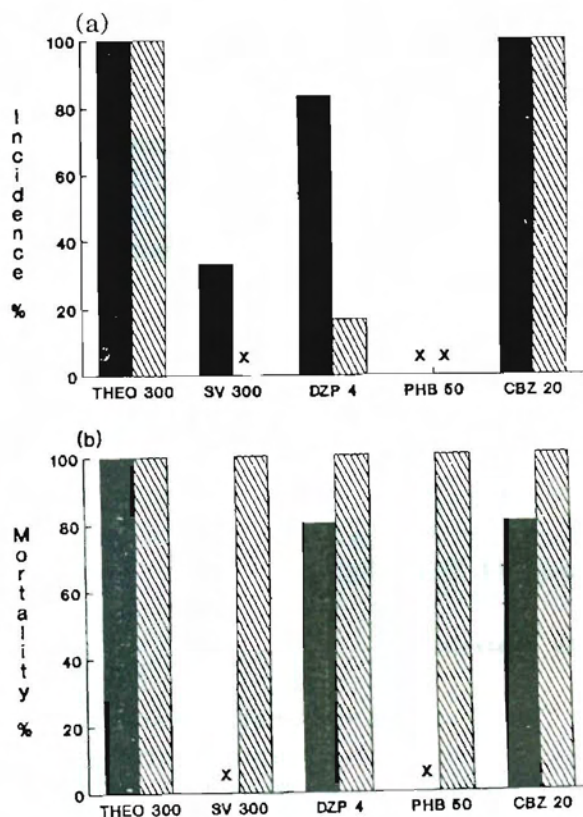


Fig. 2 : Effect of antiepileptic drugs, diazepam (DZP), sodium valproate (SV), phenobarbitone (PHB) and carbamazepine (CBZ) against theophylline (THEO)-induced seizures. (a) Effect on percent incidence of generalized clonic seizures (solid columns) and tonic hind limb extension (hatched columns), (b) Effect on percent mortality during one hour (solid columns) and in twenty four hours (hatched columns).

TABLE II : Effect of pretreatment with diazepam (DZP), sodium valproate (SV), carbamazepine (CBZ) and phenobarbitone (PHB) on latencies in seizures induced by 100% convulsant dose of theophylline (300 mg/kg). Values are mean  $\pm$  S.E.M.

S.No.	Drug & Dose (mg/kg)	Pretreatment time (min)	n	Latencies (min)	
				Clonic seizures	Hind limb extension
1.	Control	—	12	8.94 $\pm$ 1.31	11.44 $\pm$ 1.90
2.	DZP 4	60	12	13.32 $\pm$ 3.04	26.13 *
3.	SV 300	15	12	19.00 $\pm$ 1.86	0.00**
4.	PHB 50	60	12	0.00**	0.00**
5.	CBZ 20	30	12	11.11 $\pm$ 0.75	0.00**

\*No. of animals showing hind limb extension insufficient to calculate S.E.M.

\*\*None of the animals showed seizures.

change either in the incidence of seizures or the associated mortality. With adenosine 100 mg/kg, an increase in latencies of different components and decrease in incidence of hind limb tonic extension was observed. Though there was no mortality during the one hour observation period however, all animals died during the next 24 hours. With the specific adenosine A<sub>1</sub> receptor agonist, CPA, there was no significant change in the incidence of different components of theophylline-induced seizures, at the doses tested i.e. 1, 5 and 10 mg/kg. The latencies and percent mortality as compared to theophylline control animals also remained unchanged (Fig. 1 and Table 1).

*Effect of diazepam, sodium valproate, phenobarbitone and carbamazepine, on theophylline-induced seizures:*

Fig. 2 shows the change in percent incidence and mortality with the antiepileptic drugs administered before theophylline challenge. The corresponding changes in latencies are shown in Table II. Diazepam 4 mg/kg, afforded only partial protection. The incidence of tonic hind limb extension was decreased to 16.6%. Sodium valproate 300 mg/kg decreased the incidence of clonic seizures and in the animals showing clonic activity, the latency was increased. In addition, none of the valproate treated animal showed tonic hind limb extension. In phenobarbitone 50 mg/kg treated rats, none of the animal experienced either clonic or tonic seizures. Carbamazepine 20 mg/kg had no effect on theophylline-induced seizures except for abolishing the hind limb tonic extension. Notably, in sodium valproate and

phenobarbitone treated animals, there was no mortality during the observation period. However, all animals were found dead at 24 hours.

## DISCUSSION

Theophylline has been used to treat acute bronchial asthma and the acute exacerbation of chronic obstructive pulmonary disease (COPD), usually as the ethylenediamine complex, aminophylline. It however, has a narrow therapeutic window and life threatening toxicities are well documented. One of the serious toxicity of theophylline, in humans is seizures which are frequently resistant to treatment and often cause death (4-6).

In experimental animals, non-convulsive doses of methylxanthines have been shown to potentiate the convulsant effect of many chemoconvulsants e.g. pentylenetetrazole, kainic acid or pilocarpine (15, 16). Concurrent administration of methylxanthines (in subconvulsant doses) also impairs the antiepileptic activity of the antiepileptic drugs like diazepam, sodium valproate and carbamazepine *in vivo* (14, 17, 18). While, ECT induced seizures are enhanced in patients on theophylline (19).

Multitude of mechanisms have been implicated in the action of xanthines. These are phosphodiesterase (PDE) inhibition and thereby increase in cAMP levels, calcium mobilization and adenosine receptor antagonism (1, 20). More recently, methylxanthines have also been shown to interact at GABA-benzodiazepine receptor site (21), dose-dependently effect the regional release of catecholamines,

especially dopamine in the central nervous system (22) and augment release of excitatory amino acids (23). However, the CNS excitatory actions have been ascribed mainly to adenosine antagonistic properties (24, 25).

In the present study, we used adenosine and since the anticonvulsant effect of adenosine has been shown to be  $A_1$  receptor mediated (12), a highly potent and specific adenosine  $A_1$  receptor agonist, CPA, (26) was also used. Interestingly, both these agents, in the doses used, failed to afford any significant protection against theophylline seizures. Higher doses themselves had prominent behavioural/sedative effects and were therefore not used. Both adenosine and CPA in these doses have been shown to afford significant protection against pentylenetetrazole-induced seizures (12). There was a decrease in the incidence of hind limb tonic extension with adenosine 100 mg/kg which was reversed with higher doses. Also, there was no change in mortality, an important end point in theophylline-induced seizures. Such a thing was however not observed with CPA. Dunwiddie and Worth, (27), and Shanon and Maher, (28), in their studies also did not observe any protection with adenosine and non specific adenosine receptor agonists, against theophylline-induced seizures. Hornfeldt and Larson, (29) have also demonstrated, in mice, that pretreatment

with cyclohexyl adenosine, an adenosine  $A_1$  receptor agonist and dipyridamole an adenosine uptake blocker failed to inhibit the ability of theophylline to cause tonic seizures. Morgan and Durcan, (30) have rather shown a proconvulsant effect of both peripherally and centrally administered N-ethylcarboxamidoadenosine (NECA), an adenosine agonist, in caffeine-induced seizures, in mice.

Interestingly, in the present study, while both adenosine and CPA were ineffective, diazepam, sodium valproate and phenobarbitone, but not carbamazepine showed protection against theophylline seizures. Such a protection has also been documented by other workers (31). But the mortality at 24 hours remained unaffected. This mortality may perhaps be attributable to other side effects of theophylline e.g. cardiovascular.

These observations however appear contrary to the notion that theophylline seizures are mostly refractory to antiepileptic drugs in humans. Perhaps higher doses are required to elicit the protection or the observed protection may be due to species variation.

To conclude, it is apparent from this study that adenosinergic mechanisms are unlikely to be responsible for the convulsant action of theophylline and alternate mechanisms need to be explored.

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