

EFFECT OF THEOPHYLLINE ON DIAZEPAM AND SODIUM VALPROATE PROTECTION IN PENTYLENETETRAZOLE - KINDLED SEIZURES IN RATS

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Abstract : Theophylline is well known for its convulsant and proconvulsant action. Some experimental studies also suggest that theophylline and other methylxanthines may impair the protection of antiepileptic drugs. The interaction of theophylline and the antiepileptic drugs diazepam and sodium valproate was studied in pentylenetetrazole (PTZ) - kindled seizures in rats. Pretreatment with both diazepam 4 mg/kg and sodium valproate 300 mg/kg, i.p., showed protection against PTZ kindled seizures. Theophylline, 50 mg/kg, i.p., when given before the antiepileptic drugs, failed to reverse their protection. Since theophylline has an adenosine receptor antagonist activity which may be responsible for its convulsant potential, the results indicate non-involvement of adenosinergic mechanisms in the mechanisms of actions of these antiepileptic drugs.

Key words : pentylenetetrazole kindling
sodium valproate

diazepam
theophylline

INTRODUCTION

Theophylline, a methylxanthine has been used widely to treat acute bronchial asthma and the acute exacerbation of chronic obstructive pulmonary disease (COPD). The drug has narrow therapeutic index and one of its most serious toxicity in humans is seizures (1-5). Though the mechanism of bronchodilation by theophylline is multifactorial and complex, adenosine receptor antagonistic activity is believed to, play a role (6). Recently, the adenosinergic system has been suggested to be involved

in convulsions and adenosine and adenosine A₁ receptor agonists have been shown to exert an anticonvulsant effect (7, 8). That the convulsant potential of theophylline is related to its adenosine receptor antagonist activity has also been suggested (9, 10). Furthermore, in experimental animals, concurrent administration of theophylline, aminophylline and caffeine in sub-convulsant doses has been shown to impair the anticonvulsant activity of the antiepileptic drugs, diazepam, sodium valproate and carbamazepine (11,12). We have also demonstrated a reversal of

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protection of diazepam and sodium valproate, by subconvulsant doses of theophylline, in seizures induced by acutely administered pentylenetetrazole (13).

In the present study, the interaction potential of theophylline with diazepam and sodium valproate was studied, in a chronic model of generalised seizures i.e. seizures kindled by repeated administration of pentylenetetrazole (PTZ kindling), in rats.

METHODS

Experimental animals: All studies were conducted on 'Wistar' rats weighing 150-200 g. Only male rats were used. The animals were obtained from 'Central Animal Facility' of All India Institute of Medical Sciences and 'stock bred' in the departmental animal house. 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.

Pentylenetetrazole (PTZ) kindling in rats: Pentylenetetrazole (Sigma, U.S.A.) was dissolved in saline. Kindled seizures were induced by intraperitoneal injection of subconvulsant doses of PTZ i.e., 30 mg/kg, in rats, on alternate days, three times a week. The rats were observed for a period of 30 min after subconvulsant PTZ both manually and using an indigenously designed seizure recording assembly (14)

TABLE I: Scoring system for pentylenetetrazole-kindled seizures.

Stage	Symptoms
0	No Change
1	Hyperactivity, restlessness, vibrissae twitching
2	Head nodding, head clonus, myoclonic jerks
3	Unilateral or bilateral limb clonus
4	Forelimb clonic seizures
5	Generalized clonic seizures with loss of righting reflex

and seizure activity scored using a scoring system from stages 0 to 5 (Table I).

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), on 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.

All drugs were prepared freshly 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.

Experiments with diazepam and sodium valproate: Diazepam (Courtesy Intas Laboratories Ltd., India), was suspended in 3% tween 80 and administered in a dose of 4 mg/kg, 60 min before challenge with subconvulsant dose of PTZ. 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 PTZ challenge.

The pretreatment timings of the drugs were decided on the basis of their peak activity and the doses selected are those shown to be 100% protective against seizures induced by acute administration of convulsant dose of PTZ (60 mg/kg) in experiments in our laboratory setup (13).

Effect of theophylline pretreatment on the protection of diazepam and sodium valproate: Theophylline (Courtesy Sun Pharma, India), was dissolved in warm saline and administered intraperitoneally, in a dose of 50 mg/kg before the antiepileptic drug treatment.

Statistical analysis of data: The results were analysed statistically using a microsoft computer programme, 'microstat' copyright Ecosoft Inc., U.S.A.

RESULTS

Establishment of PTZ - kindled seizures in rats: Development of fully kindled, stage 5 seizures i.e. generalised clonic seizures with loss of righting reflex in rats occurred after approximately 10 weeks of treatment with subconvulsant doses of pentylenetetrazole. That the change is permanent was established by rechallenge with PTZ on the 3rd and 10th day after stopping PTZ treatment in these rats. The progression from normal to stage 5 seizures, in rats during the course of kindling is shown in Fig. 1. The mortality during induction of kindling was low (10%). The mean latency to the stage 5 seizures i.e. generalized clonic seizure with loss of righting reflex was 2.74 ± 0.92 min.

Interaction of theophylline with diazepam and sodium valproate in PTZ - kindled rats: With both diazepam 4 mg/kg, 60 min pretreatment as well as sodium valproate 300 mg/kg, 15 min pretreatment, a 100% protection against PTZ kindled seizures was observed i.e. none of the animals experienced any seizure activity.

Theophylline *per se* in doses of 25, 50, 100, 150 and 200 mg/kg did not cause any seizure activity in rats. Only at higher doses i.e. 250, 275 and 300 mg/kg, it caused generalized clonic seizures, hind limb tonic extension and mortality was observed (data not shown). Similarly, theophylline 50 and 100 mg/kg did not aggravate PTZ-induced seizures. This dose could however reverse the protection of both adenosine and the adenosine A₁ receptor agonist, N⁶-cyclopentyladenosine (CPA), in PTZ-induced seizures (7).

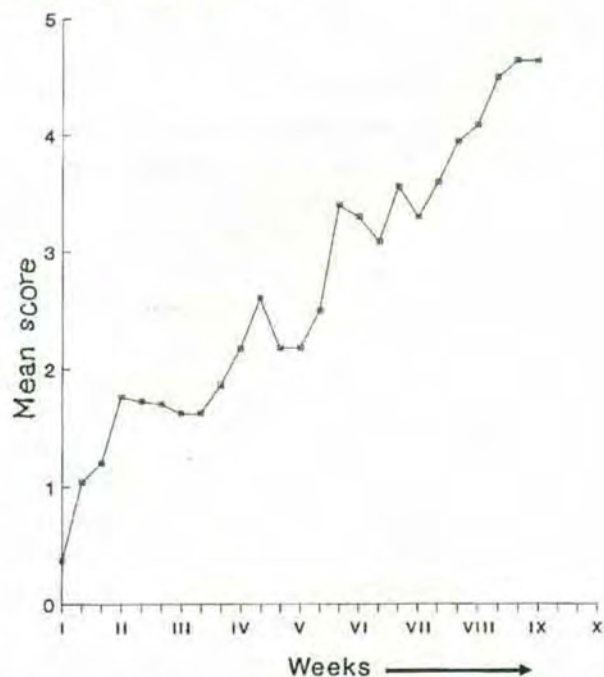


Fig. 1: Establishment of pentylenetetrazole-kindled seizures in rats.

In PTZ kindled seizures, pretreatment with theophylline, 50 mg/kg, i.p. 5 and 30 min before the protective doses of diazepam and sodium valproate respectively, however failed to significantly reverse the protection of these drugs in this model of seizures. The mean scores were 0.00 and 1.6 ± 1.05 respectively.

DISCUSSION

In the present study, theophylline 50 mg/kg, failed to reverse the protection of both diazepam as well as sodium valproate in PTZ-kindled seizures. This is contrary to the expectations based on the previous reports where theophylline and other methylxanthines eg. aminophylline and caffeine have been shown to impair the anticonvulsant efficacy of anticonvulsant drugs in mice and rats. However, the experimental models of seizures used in these studies have been different i.e., electroconvulsions (11), pentylenetetrazole-induced seizures (12, 13) and amygdala kindling (15). To our knowledge, there is no report on the interaction of methylxanthines and the antiepileptic drugs in PTZ kindled seizures.

PTZ kindled seizures are an animal model of generalized epilepsy (16). These seizures are characterized by generalized spike and wave discharges on EEG, concomitant with generalized seizures such as myoclonic and tonic seizures. The basic mechanisms involved in PTZ kindled seizures are unknown but since PTZ acts via a specific interaction with GABA-coupled chloride ionophore (17), therefore, the role of GABAergic neurons is a possibility. Interestingly enough, in the present study, reversal of protection in PTZ kindled seizures was not observed though in acute PTZ administration induced seizures, the anticonvulsant efficacy of diazepam and sodium valproate was reversed (13). This

observed difference can only be accounted for by the dissimilar mechanisms involved in the two models of epilepsy. Difference in the mechanism of chronic epileptogenesis (development of kindling induced seizure susceptibility) and those of the acute convulsive reaction to the epileptogen has been reported (18).

The fact that other workers have shown reversal of antiepileptic drug protection in amygdala kindled seizures suggests that different mechanisms may be involved in these two models of chronic epileptogenesis. It is conceivable that different kindling stimuli bring about different series of interactions between multiple neuronal systems, eventually resulting in the common behavioral expression of generalized seizures (19, 20).

It has been suggested that the convulsant and proconvulsant action of theophylline and other methylxanthines may be related to the adenosine receptor antagonist activity (9, 10). The dose of theophylline used in this study is sufficient for adenosine antagonist activity since as mentioned before, this dose (50 mg/kg) could completely reverse the protection of both adenosine and adenosine A_1 receptor agonist, N^6 -cyclopentyl adenosine (CPA) in PTZ-induced seizures in our earlier experiments (7). Other workers have also used this dose of theophylline for studying adenosine receptor antagonism (21). Thus the non reversal of protection by theophylline, in this model may be attributed either to non involvement of adenosinergic mechanisms in the anticonvulsant action of the antiepileptic drugs diazepam and sodium valproate or the non-involvement of adenosinergic mechanisms in PTZ kindled seizures.

Of these two possible explanations, the

latter appears to be more likely because in our experiments with adenosinergic agents in PTZ kindled seizures, no significant protection was observed (7).

The findings thus suggest that though

adenosinergic system is reported to be involved in the seizure activity, the neurochemical mechanisms in different convulsions are not similar and the adenosinergic mechanisms are not involved in PTZ kindled seizures.

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