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Adenosine Receptor Antagonism Accounts for the Seizure-Prolonging Effects of Aminophylline[1]

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DRAGUNOW, M. *Adenosine receptor antagonism accounts for the seizure-prolonging effects of aminophylline.* PHARMACOL BIOCHEM BEHAV **36**(4) 751–755, 1990.—The mechanism of action of aminophylline in prolonging seizures was tested in amygdala-kindled rats. Aminophylline prolonged the afterdischarge duration of kindled seizures. This seizure-prolonging action of aminophylline was strongly antagonized by the adenosine A1 agonist cyclohexyladenosine and partially antagonized by the benzodiazepine partial agonist RO 15-1788. However, the specific benzodiazepine antagonist CGS 8216 did not affect the seizure-prolonging action of aminophylline. Also, the potent anticonvulsant effect of diazepam on kindled seizures, which was completely antagonized by CGS 8216, was unaffected by aminophylline. Furthermore, a range of benzodiazepine inverse agonists, GABA antagonists, phosphodiesterase inhibitors and xanthines did not prolong afterdischarge durations. These results demonstrate that the seizure-prolonging action of aminophylline is due to block of A1 adenosine receptors since it is prevented by adenosine A1 agonists.

Aminophylline Kindling Seizure duration

THE methylxanthine adenosine antagonists caffeine and theophylline prolong kindled seizures in rats (1, 2, 4, 8, 10, 11) and ECT seizures in humans (24,27). These observations, taken together with other data, have led to the hypothesis that endogenous adenosine terminates seizures (12,13) and that status epilepticus and associated cell death may involve a perturbation of this anticonvulsant system (12,13). Caffeine and theophylline have other neurochemical actions besides adenosine antagonism [e.g., effects on cyclic nucleotides by inhibiting phosphodiesterase and benzodiazepine receptors, (10)], and these effects could account for their seizure-prolonging action. In this paper a series of experiments are described which are aimed at testing the relative contributions of adenosine receptor antagonism, benzodiazepine receptor blockade and cyclic nucleotides in theophylline's seizure-prolonging action.

In these experiments a water soluble complex containing 80% theophylline and 20% ethylenediamine, called aminophylline, was used. It was tested on amygdala-kindled seizures in rats because previous studies have shown that it prolongs the afterdischarge duration (ADD) of these seizures (10). We tested the effects of the adenosine agonist cyclohexyladenosine (CHA) and the benzodiazepine antagonists RO 15-1788 (Flumazenil) and CGS 8216 (3) on this seizure-prolonging action of aminophylline. We tested whether aminophylline would reverse the anticonvulsant effects of diazepam on kindled seizures as has been reported in other seizure models (25). Finally, we investigated if various drugs would mimic the seizure-prolonging action of aminophylline. These drugs were the GABA antagonist picrotoxin, the benzodiazepine inverse agonists FG 7142 and DMCM, the phosphodiesterase inhibitor rolipram (29), the propyl xanthine enprofylline [which has weak central adenosine antagonistic effects, (23)] and the specific and potent adenosine antagonist 8-phenyltheophylline [8PT, (6,9)].

METHOD

Drugs

Aminophylline (David Bull labs) was dissolved in saline at a concentration of 25 mg/ml. Cyclohexyladenosine (CHA, Boehringer-Mannheim) was dissolved in saline. RO 15-1788 (Hoffmann-La Roche), CGS 8216 (CIBA-GEIGY), DMCM (Schering), rolipram (Schering), FG 7142 (Ferrosan) and Enprofylline (AB Draco) were suspended in saline containing Tween 80 and subjected to 10 minutes of ultrasound treatment. Diazepam (Roche) was dissolved in its proprietary vehicle. 8PT (Research Biochemicals) was dissolved in a drop of 95% ethanol and brought up to volume with propylene glycol.

Surgical and Kindling Procedure

Adult male Sprague-Dawley rats were anaesthetized with

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TABLE 1

DRUG INJECTION PROTOCOL

Drug 1			Drug 2		
Drug	Dose (mg/kg)	Injection Time (min)	Drug	Dose (mg/kg)	Injection Time (min)
Vehicle	—	30	Vehicle	—	30
Aminophylline	50	30	Vehicle	—	30
Aminophylline	50	30	CHA	2	30
Aminophylline	50	30	CHA	4	30
Aminophylline	50	30	RO 15-1788	10	10
Vehicle	—	30	RO 15-1788	10	10
Aminophylline	50	30	CGS 8216	20	30
Aminophylline	50	30	CGS 8216	40	30
Aminophylline	50	30	CGS 8216	80	30
Vehicle	—	30	CGS 8216	40	30
Vehicle	—	30	Diazepam	2	15
Aminophylline	50	30	Diazepam	2	15
CGS 8216	20	30	Diazepam	2	15
FG 7142	0, 2.5, 5, 10	10	—	—	—
DMCM	0, 0.5, 1, 2, 4	5	—	—	—
Rolipram	0, 12.5, 25, 50, 100	30	—	—	—
Enprofylline	0, 10, 50, 100	45	—	—	—
8PT	0, 5, 10, 20	30	—	—	—
Picrotoxin	0, 1	20	—	—	—

Injection time refers to the number of minutes the drug was injected prestimulation.

barbiturate and stereotaxically implanted in the right amygdala (AP 0.0 mm, L 4.8 mm, V 9.0 mm from skull surface, incisor bar 5 mm above interaural line, bregma = zero) with bipolar stainless steel electrodes (Plastic Products MS 333/2-B). A third electrode lead served as an indifferent electrode for EEG recording and was connected to a skull screw positioned above the right cortical surface. Three additional skull screws and dental cement served to anchor the electrode in position. At least two weeks elapsed for postsurgical recovery before any experimentation began. All rats were housed individually with ad lib food and water in a room with reverse light cycle.

Kindling was by electrical stimulation (WPI constant current stimulator) to the right amygdala once per day with 2 seconds, 100 Hz, 300 μA peak-to-peak, 0.5 msec/half-wave biphasic pulses. The EEG from the amygdala was recorded from between the poles of the stimulating electrodes and between one pole of the stimulating electrode and the skull screw indifferent electrode on a Grass 79D EEG. The motor seizures were rated as follows: stage 0, no response or behavioural arrest; stage 1, rhythmic mouth and facial movements; stage 2, rhythmic head nodding; stage 3, forelimb clonus; stage 4, rearing with bilateral forelimb clonus; and stage 5, rearing and falling preceded by all of the above. Once animals reached the kindling criterion of 3 consecutive stage 5 seizures they were given at least 2 weeks without treatment and were then used in the drug studies.

Drug Studies

Amygdala-kindled rats were randomly assigned to various groups as detailed in Table 1. All drugs were injected IP. At various times after injection (as detailed in Table 1), rats were connected up to the EEG and stimulation apparatus and were stimulated for 2 sec with 100 Hz, 300 μA peak-to-peak, 0.5 msec/half-wave biphasic pulses. Some rats were used in more than one pharmacological test. In such cases rats were always given at least a 2-week "rest" period between treatments. However, each drug group also contained drug-naive rats that had not been previously used in experiments. A comparison was made of the seizure scores for rats reused in experiments and drug-naive rats for each drug group and there were no significant differences in their seizure scores.

Histology

At the completion of the drug experiments randomly selected rats from every drug group were administered a barbiturate overdose and perfused transcardially with 10% formalin solution. Electrode locations were checked using a light microscope after sectioning the brains coronally, mounting selected sections on microscopic slides, and Nissl staining them with thionin. In all the rats randomly selected for histology, electrodes were located in the amygdala.

RESULTS

Aminophylline (50 mg/kg, IP) prolonged the afterdischarge duration (ADD) of amygdala-kindled seizures (Table 2). This seizure-prolonging action of aminophylline was antagonized by CHA at 2 mg/kg, and completely blocked by 4 mg/kg CHA (Table 2). CHA given alone (2 mg/kg) did not significantly reduce ADD at these drug doses (mean ADD = 80.1 ± 12.3).

RO 15-1788 (10 mg/kg) also antagonized the seizure-prolonging action of aminophylline, but also had anticonvulsant effects on amygdala-kindled seizures when administered alone at this dose of 10 mg/kg (Table 2). CGS 8216 in doses from 20–80 mg/kg did not modify the seizure-prolonging action of aminophylline (Table 2) and had no anti- or proconvulsant effects on amygdala-kindled seizures when injected alone (Table 3). Diazepam (2 mg/kg) completely blocked the generalization of amygdala-kindled seizures and profoundly reduced ADD (Table 3). This

TABLE 2

THE EFFECTS OF CHA (2 AND 4 mg/kg), RO 15-1788 (10 mg/kg) AND CGS 8216 (20, 40 AND 80 mg/kg) ON THE SEIZURE PROLONGING ACTION OF AMINOPHYLLINE ON AMYGDALA-KINDLED SEIZURES

Drug Treatment	N	Afterdischarge Duration (sec) (mean ± sd)	*t*-Value	*df*	*p*
Vehicle + Vehicle	5	89.6 ± 23.8	—	—	—
Aminophylline + Vehicle	6	228.7 ± 44.9	6.2	9	0.001
Aminophylline + CHA (2)	7	113.0 ± 37.7	1.2	10	NS
Aminophylline + CHA (4)	9	71.3 ± 27.7	1.2	12	NS
Aminophylline + RO 15-1788	11	141.0 ± 65.0	1.6	14	NS
Vehicle + RO 15-1788	5	40.6 ± 34.5	2.6	8	0.03
Aminophylline + CGS 8216 (20)	8	195.0 ± 99.4	2.3	11	0.04
Aminophylline + CGS 8216 (40)	12	180.5 ± 49.3	3.9	15	0.01
Aminophylline + CGS 8216 (80)	5	212.0 ± 66.1	3.9	8	0.04

Unpaired *t*-tests were carried out for each drug treatment versus the Vehicle + Vehicle control group (NS, not significantly different).

anticonvulsant effect of diazepam was antagonized by CGS 8216 (20 mg/kg), but not affected by aminophylline (50 mg/kg) (Table 3).

FG 7142 (2.5–10 mg/kg) did not prolong amygdala-kindled seizures (Table 4) in those rats that were not having FG 7142-induced seizures. However, 3 of the 6 amygdala-kindled rats injected with 10 mg/kg FG 7142 went rapidly (<5 min) into spontaneous recurrent convulsions (stage 5). Two of the six rats showed myoclonic jerking. Three of three amygdala-kindled rats injected with 20 mg/kg FG 7142 also had spontaneous recurrent tonic/clonic convulsions. However, in 4 nonkindled rats FG 7142 (20 mg/kg) failed to elicit any seizures. DMCM (0.5–4.0 mg/kg) also failed to prolong ADD of amygdala-kindled seizures (Table 4) in those rats that did not have spontaneous recurrent tonic/clonic seizures. Also, picrotoxin did not prolong ADD (Table 4).

Rolipram did not prolong the ADD of amygdala-kindled seizures (Table 4), or produce seizures (at 200 mg/kg). Enprofylline produced ataxia but did not prolong kindled seizures (Table 4). 8PT did not affect ADD of amygdala-kindled seizures at any dose (Table 4).

DISCUSSION

Aminophylline greatly prolonged the duration of amygdala-kindled seizures in rats. This result is consistent with previous observations (1, 2, 8, 10, 11, 21). Similarly, aminophylline has been shown to prolong cortical (1), perforant-path (4) and hippocampal seizures (7,15). CHA blocked this seizure-prolonging action of aminophylline at doses that were not anticonvulsant when given alone (30). These results suggest that aminophylline prolongs kindled seizures by blocking adenosine receptors.

The seizure-prolonging action of aminophylline was also antagonized by RO 15-1788, a benzodiazepine partial agonist (26), however, this antagonism occurred at doses that were themselves anticonvulsant. Previous studies have shown that RO 15-1788 has a weak anticonvulsant effect on kindled seizures (26) and we have replicated this observation. However, CGS 8216, a specific benzodiazepine antagonist, did not antagonize the effects of aminophylline supporting previous results (5) and aminophylline did not prevent the anticonvulsant effects of diazepam. Furthermore, picrotoxin, FG 7142 and DMCM, at subconvulsant doses, did not prolong kindled seizures. Thus, it seems clear that the seizure-prolonging action of aminophylline does *not* involve benzodiazepine systems. The partial antagonism of aminophylline's proconvulsant effect by RO 15-1788 more likely reflects antagonism by a weak anticonvulsant. Alternatively, the interaction of RO 15-1788 with aminophylline as well as its direct anticonvulsant action might reflect the observation that RO 15-1788 is an adenosine uptake blocker (28).

TABLE 3

THE EFFECTS OF AMINOPHYLLINE (50 mg/kg) AND CGS 8216 (20 mg/kg) ON THE AFTERDISCHARGE SHORTENING ACTION OF DIAZEPAM (2 mg/kg) ON AMYGDALA-KINDLED SEIZURES

Drug Treatment	N	Afterdischarge Duration (sec) (mean ± sd)	*t*-Value	*df*	*p*
Vehicle + Vehicle	6	117.2 ± 32.8	—	—	—
Diazepam + Vehicle	6	20.5 ± 13.9	6.07	10	0.01
Diazepam + CGS 8216	3	68.7 ± 6.2	2.2	7	NS
Vehicle + CGS 8216	4	99.3 ± 6.9	1.0	8	NS
Diazepam + Aminophylline	6	23.5 ± 13.3	5.9	10	0.01

Unpaired *t*-tests were carried out for each drug treatment versus the Vehicle + Vehicle control group (NS, not significant).

TABLE 4

THE EFFECTS OF PICROTOXIN, FG 7142, DMCM, ROLIPRAM, ENPROFYLLINE, AND 8PT ON THE AFTERDISCHARGE DURATION OF AMYGDALA-KINDLED SEIZURES

Drug	Dose (mg/kg)	N	Afterdischarge Duration (sec) (mean ± sd)	F-Value	*df*	*p*
Picrotoxin	0	5	98.6 ± 21.8			
	1	5	113.9 ± 21.5	($t=1.0$)	(8)	NS
FG 7142	0	3	88.2 ± 33.4			
	2.5	4	92.2 ± 40.5			
	5.0	4	71.5 ± 43.1			
	10.0	6	112.2 ± 57.1	0.7	3,14	NS
DMCM	0	5	106.2 ± 38.0			
	0.5	3	98.7 ± 17.8			
	1.0	4	127.0 ± 24.6			
	2.0	4	105.3 ± 45.6			
	4.0	5	91.4 ± 72.6	0.3	4,16	NS
Rolipram	0	10	99.8 ± 18.5			
	12.5	9	104.7 ± 15.2			
	25.0	12	121.8 ± 41.6			
	50.0	7	122.4 ± 41.6			
	100.0	2	80.0 ± 14.1	1.7	4,35	NS
Enprofylline	0	3	96.5 ± 69.9			
	10	4	126.5 ± 60.0			
	50	4	54.0 ± 29.3			
	100	2	(13,39)	1.6	3,10	NS
8PT	0	16	78.4 ± 41.3			
	5	10	74.4 ± 75.6			
	10	6	139.5 ± 73.3			
	20	6	115.3 ± 64.2	2.1	3,34	NS

One-way analysis of variance was performed for each drug except for the picrotoxin where an unpaired *t*-test was used.

Because CHA blocked the seizure-prolonging action of aminophylline, it seems very likely that aminophylline is working through adenosine systems. This hypothesis is supported by the observations that the phosphodiesterase inhibitor rolipram (23) and the weak adenosine antagonist enprofylline (29) did not prolong seizures. However, the specific, potent and A1 selective adenosine antagonist 8PT did not prolong kindled seizures. However, although 8PT is potent in in vitro preparations (8), there is good evidence to suggest that it lacks in vivo adenosine antagonistic ability. Hence, Fredholm *et al.* (20) found that after IP injection (the same route used here) to rats, 8PT did not enter the brain or plasma in detectible amounts and did *not* mimic the effects of theophylline on dopamine-induced turning behaviour in rats. Thus, because of the hydrophobic nature of this phenyl derivative, it may not be adequately absorbed into the circulation after IP injection. On the other hand, 8-cyclopentyltheophylline, an A1 selective adenosine antagonist (6) which has physicochemical properties suggestive of in vivo activity (6), mimics the action of theophylline in facilitating hippocampal afterdischarges (14).

In conclusion, the results of this study show that the seizure-prolonging action of aminophylline is blocked by an adenosine A1 receptor agonist but not by a benzodiazepine antagonist. These observations, together with other results showing that drugs sharing aminophylline's other neurochemical effects (i.e., benzodiazepine blockade, phosphodiesterase inhibition) do not prolong seizures, suggest that the seizure-prolonging action of aminophylline is caused by its antagonism of A1 adenosine receptors which play a physiological role in seizure arrest (10–19).

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