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A Comparative Study of Aminophylline- and Acepifylline-induced Seizures and Death in the Chemoconvulsion Model in Rats

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Abstract

The convulsive, pro-convulsive and lethal effects of two theophylline-containing bronchodilating agents, aminophylline and acepifylline, have been evaluated in rats.

Aminophylline (theophylline ethylenediamine) caused seizures and death in a dose-dependent manner; an intraperitoneal dose of 250 mg kg^{-1} caused seizures and death in all rats. Intraperitoneal doses of acepifylline (theophylline ethanoate of piperazine) up to 1000 mg kg^{-1}, however, did not cause seizure or death. Further, pre-treatment of the rats by intraperitoneal administration of a subconvulsive dose (100 mg kg^{-1}) of aminophylline caused a significant decrease in CD50 and LD50 values for pentylenetetrazole and a significant increase in the number of positive responders (i.e. rats with a pentylenetetrazole-induced seizure score of 3 or more on a seizure scale ranging from 0 to 6) and death rate compared with those obtained for rats pre-treated with an equivalent intraperitoneal dose (140 mg kg^{-1}) of acepifylline ('equivalent dose' referred to here denotes the theophylline content of the two preparations).

The study has established the neurosafety profile of acepifylline and documents a safer alternative to aminophylline for use in asthmatics suffering from concomitant epilepsy or other seizure-prone neurological defects.

Aminophylline (theophylline ethylenediamine) is a widely used bronchodilator with a narrow effective therapeutic range. In contrast, acepifylline (theophylline ethanoate of piperazine) is prescribed rather sparingly for similar conditions. Schwartz & Scott (1975) reported that toxic concentrations of theophylline caused repetitive generalized seizures in asthmatic patients and Chugh et al (1991) have reported that a single dose of aminophylline (240 mg kg^{-1}, i.p.) caused reproducible tonic-clonic seizures and death in mice. Further, the neurosensitizing potential of aminophylline was documented in electroshock models of seizures in mice by Chakrabarti et al (1993). However, no such study pertaining to the neurotoxicity of, or neurosensitization with, acepifylline has been reported in the literature.

This study was designed to compare the neurotoxic and neurosensitizing potential in rats of aminophylline and acepifylline (each of which contains theophylline as the principal ingredient).

Materials and Methods

Animals

Experiments were performed on adult male Sprague–Dawley rats, 100–150 g. The animals were kept in colony cages at an ambient temperature of 25 ± 1°C, a relative humidity of 60 ± 2%, and were exposed to a 12-h light–dark cycle. They had free access to food (Hindustan Lever chow) and tap-water. The experimental groups consisted of 8 to 10 rats.

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Drugs and chemicals

Pentylenetetrazole (Lot 74HO384) was from Sigma (USA). Aminophylline injection in ampoules containing 25 mg mL^{-1} (batch number AP-66) was from G&G Pharmaceuticals, Punjab, India. Acepifylline powder (batch number CL 30723) was from Cadila Chemicals Limited, Ahmedabad, India.

Aminophylline-induced seizures

Experiments were performed with graded, single doses (100 to 250 mg kg^{-1}) of aminophylline administered intraperitoneally (i.p.) to five groups of rats. The rats were observed for 1 h for the occurrence of frank convulsions (i.e. tonic hind limb extension) and death. The tests were performed in a quiet room.

Acepifylline-induced seizures

Experiments were performed as stated above with graded, single doses (350, 700, 800 or 1000 mg kg^{-1}, i.p.) of acepifylline administered to four groups of rats. All drug solutions were freshly prepared before use.

Pentylenetetrazole-induced seizures in rats pre-treated with saline

Pentylenetetrazole solution (0·75% in 0·9% NaCl) was injected intraperitoneally in graded, single doses (30 to 120 mg kg^{-1}) to seven groups each of 10 rats in individual perspex cages (25 × 25 × 10 cm). They were observed for 1 h for recording of seizure score and death; the seizure score was recorded according to the scale: 0, no response; 1, ear and facial twitching; 2, 1 to 20 myoclonic body jerks in 10 min; 3, more than 20 body jerks in 10 min; 4, clonic forelimb convulsions;

5, generalized clonic convulsions with episodes of rearing and falling down; 6, generalized epilepticus. The response to pentylenetetrazole was considered positive when the seizure score was ≥ 3 (Giorgi et al 1991).

Pentylenetetrazole-induced seizures in rats pre-treated with aminophylline

Rats were pre-treated with a single subconvulsive dose (100 mg kg^{-1}, i.p.) of aminophylline and observed for any behavioural alterations, seizure or death. Two hours later pentylenetetrazole was administered in graded, single doses (10 to 50 mg kg^{-1}, i.p.) to five groups of rats and the rats were observed for 1 h for recording of seizure score and death.

Pentylenetetrazole-induced seizures in rats pre-treated with acepifylline

Rats were pre-treated with acepifylline (140 mg kg^{-1}, i.p.; equivalent to approximately 100 mg kg^{-1} aminophylline in terms of theophylline content). Two hours later, pentylenetetrazole was administered in graded, single doses (20 to 100 mg kg^{-1}, i.p.) to seven groups of rats and the rats were observed for 1 h for recording of seizure score and death as described above.

Statistics

Data for positive responders and death were compared by Fisher's exact probability test. Pentylenetetrazole CD50 values (i.e. the dose causing positive seizure response in 50% of rats) and LD50 values (i.e. the dose lethal to 50% of rats) were determined by the graphic method. Log dose-probit lines were obtained by least-squares regression analysis. The standard error (s.e.m.) of CD50 and LD50 values was calculated using the formulae (log CD84 – log CD16)/$2N^{-½}$ for the s.e.m. of CD50 and (log LD84 – log LD16)/$2N^{-½}$ for the s.e.m. of LD50, where N is the total number of animals in the groups which from the best fitting line would be expected to show effects (seizure or death) between the probits 3·5 and 6·5. The log values were obtained from the line on the graph corresponding to probits 6 and 4 (Ghosh 1984). The CD50 ± s.e.m. and LD50 ± s.e.m. values thus obtained for the different treatment groups were compared by Student's *t*-test. *P* values less than 0·05 were considered statistically significant.

Results

Table 1 shows the effect of administration of a graded, single dose of aminophylline to different groups of rats. When administered at an intraperitoneal dose of 100 mg kg^{-1}, aminophylline did not cause seizure or death whereas at 250 mg kg^{-1} it induced severe tonic-clonic convulsions with hind limb extension and immediate post-convulsive death in all rats. The latency to the onset of convulsions ranged from 15 to 30 min whereas the duration of convulsions varied from 1·4 to 2·3 min. Intermediate doses of aminophylline produced a graded response both for convulsions and for death. In contrast, acepifylline did not cause seizure or death over a wide dose range (350 to 1000 mg kg^{-1}, i.p.). The majority of rats treated with acepifylline showed increased respiration, mild hyperactivity and some lack of motor coordination without frank seizure or death.

Table 2 shows the effect of pre-treatment with saline or with single doses of aminophylline or acepifylline on pentylenetetrazole-induced seizures and death in rats. Pre-treatment with aminophylline of groups receiving 30, 40 or 50 mg kg^{-1} intraperitoneal pentylenetetrazole resulted in a significant increase in positive responders and death compared with the groups pre-treated with saline. The CD50 ± s.e.m. value for pentylenetetrazole was significantly lower (17·98 ± 1·15 mg kg^{-1}, i.p.) for the aminophylline pre-treated group than for the saline pre-treated group (58·21 ± 1·08 mg kg^{-1}, i.p.); the same was true for the LD50 ± s.e.m. value (29·80 ± 1·12 mg kg^{-1}, i.p., for the aminophylline pre-treated group and 78·52 ± 1·09 mg kg^{-1}, i.p., for the saline pre-treated group).

Table 1. Effect of graded doses of aminophylline on convulsions and death in rats.

Aminophylline (mg kg^{-1}, i.p.)	Convulsions (n = 8)	Deaths (n = 8)
100	0	0
150	1	1
175	5	5
200	7	7
250	8	8

Pre-treatment with acepifylline of groups receiving 50 mg kg^{-1} intraperitoneal pentylenetetrazole resulted in significantly more positive responders than for the group pre-treated with saline. At the dose levels studied there was no significant difference between the lethality to pentylenetetrazole in the groups of rats pre-treated with saline and acepifylline. Compared with the groups pre-treated with aminophylline, in the acepifylline pre-treated groups of rats the numbers of positive responders were significantly less for intraperitoneal doses of 20, 30, 40 or 50 mg kg^{-1} pentylenetetrazole and the death rate was significantly less for intraperitoneal doses of 30, 40 or 50 mg kg^{-1} pentylenetetrazole. CD50 ± s.e.m. and LD50 ± s.e.m. values for pentylenetetrazole were significantly higher for the group receiving pre-treatment with acepifylline than for that receiving pre-treatment with aminophylline and the values were significantly lower than for the group receiving pre-treatment with saline (CD50 ± s.e.m. and LD50 ± s.e.m. values for intraperitoneal pentylenetetrazole were 46·77 ± 1·08 and 69·98 ± 1·23 mg kg^{-1}, respectively for the group of rats receiving acepifylline pre-treatment (Table 2).

Discussion

These results confirmed previous reports (Czuczwar et al 1987; Chugh et al 1991) that aminophylline leads to seizure and death in a graded dose-dependent manner in rats. Intraperitoneal aminophylline at 250 mg kg^{-1} caused reproducible seizure and post-seizure death in 100% of rats. The convulsion induced by aminophylline is caused by theophylline because ethylenediamine has no effect (Chu 1981). Acepifylline, the theophylline ethanoate of piperazine, is another theophylline-containing salt. It, however, failed to cause seizure or death in rats despite the administration of massive doses (up to 1000 mg kg^{-1} i.p). Only mild stimulation of the central nervous system was observed in rats treated with the higher doses

Table 2. Effect of single-dose pre-treatment with aminophylline and acepifylline on pentylenetetrazole-induced seizures and death in rats.

Group	Pentylenetetrazole (mg kg^{-1}, i.p.)	[a]Positive responders (n = 10)	[b]CD50 ± s.e.m. (mg kg^{-1})	Deaths (n = 10)	[b]LD50 ± s.e.m. (mg kg^{-1})
Normal saline (0·5 mL, i.p., 2 h)	30	0	58·21 ± 1·08 (40)	0	78·52 ± 1·09 (30)
	40	2		0	
	50	3		0	
	60	5		1	
	75	8		3	
	100	10		8	
	120	10		10	
Aminophylline (100 mg kg^{-1}, i.p., 2 h)	10	2	17·98 ± 1·15* (30)	0	29·80 ± 1·12* (30)
	20	5		1	
	30	7*		3*	
	40	10*		7*	
	50	10*		10*	
Acepifylline (140 mg kg^{-1}, i.p., 2 h)	20	0†	46·77 ± 1·08*† (50)	0	69·98 ± 1·23*† (30)
	30	2†		0†	
	40	3†		0†	
	50	6*†		1†	
	60	7		2	
	75	9		5	
	100	10		10	

[a]Positive responders are rats with a seizure score ≥3 after administration of pentylenetetrazole. [b]Numbers in parentheses indicate the number of rats considered from the log dose-probit line for determination of s.e.m. (see text). *$P < 0·05$, significantly different from the saline-treated group; † $P < 0·05$, significantly different from the aminophylline-treated group.

of acepifylline. The exact reason for this innocuous behaviour of acepifylline in terms of neurotoxicity and consequent death was unclear. It could be because of poor penetration of acepifylline into the central nervous system of rats; this, however, needs confirmation. The aforementioned observations confirmed greater neurotoxicity with aminophylline than acepifylline. In the pentylenetetrazole model of seizures, it was observed that the clonic seizures produced by pentylenetetrazole (at 60 mg kg^{-1}, i.p. and higher doses up to 120 mg kg^{-1}, i.p.) were similar in nature to those described by Swinyard et al (1952). A subconvulsive dose of aminophylline (100 mg kg^{-1}, i.p.) had caused significant potentiation of pentylenetetrazole-induced seizure and death in rats. The CD50 ± s.e.m. and LD50 ± s.e.m. doses for pentylenetetrazole (58·21 ± 1·08 and 78·52 ± 1·09 mg kg^{-1}, i.p., respectively) were significantly reduced (to 17·98 ± 1·15 and 29·80 ± 1·12 mg kg^{-1}, respectively) after aminophylline pre-treatment. This finding highlights the strong neurosensitizing potential of aminophylline to pentylenetetrazole-induced seizures in rats, as reported previously for mice (Chugh et al 1993). Intraperitoneal pre-treatment of the rats with 140 mg kg^{-1} acepifylline also caused significant reduction in the CD50 and LD50 values for intraperitoneal pentylenetetrazole (to 46·77 ± 1·08 and 69·98 ± 1·23 mg kg^{-1}, respectively) compared with those for pentylenetetrazole alone. It should, however, be noted that whereas acepifylline reduced the CD50 and LD50 doses for pentylenetetrazole by 20% and 11% respectively, an equivalent dose of aminophylline caused reduction in the values by 69% and 62%, respectively, compared with those for pentylenetetrazole alone. The pre-treatment doses for acepifylline (140 mg kg^{-1}, i.p.) and aminophylline (100 mg kg^{-1} ,i.p.) were selected on the basis of their approximately equivalent theophylline content. It has been reported that 1·75 g acepifylline and 1·27 g aminophylline are approximately equivalent to 1 g theophylline (Wade 1977). Thus, neurosensitization to pentylenetetrazole was much more with aminophylline than with acepifylline. In clinical practice for effective bronchodilation in patients suffering from bronchial asthma or chronic obstructive pulmonary disease acepifylline can be given orally in doses of up to 1·5 g daily in three divided doses whereas the orally administered doses used for aminophylline generally range from 100 to 300 mg three or four times daily in adults. Both the drugs can also be used intramuscularly and as slow intravenous injections (Reynolds 1996).

Thus this study has confirmed the neurotoxic and neurosensitizing potential of aminophylline. It also substantiated, for the first time, that acepifylline is a much safer alternative to aminophylline particularly with regard to inducing seizures and death and causing sensitization to seizure-provoking agents in rats. The greater neurosafety profile for acepifylline here documented provides for a safer alternative to aminophylline for use in patients with chronic obstructive pulmonary disease and bronchial asthma suffering from concomitant epilepsy or other forms of seizure-prone neurological defects. It is urged that formal clinical trial with acepifylline be conducted in such patients.

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