Pharmacology Biochemistry & Behavior, Vol. 24, pp. 1599–1603, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Effect of Aminophylline on Amygdaloid-Kindled Postictal Inhibition

T. E. ALBERTSON[1]

Department of Internal Medicine, Division of Emergency Medicine and Clinical Toxicology School of Medicine, University of California, Davis CA 95616

Received 30 August 1985

ALBERTSON, T. E. *Effect of aminophylline on amygdaloid-kindled postictal inhibition.* PHARMACOL BIOCHEM BEHAV **24**(6) 1599–1603, 1986.—Previous studies have shown that methylxanthines such as aminophylline increase the clinical severity and length of electrically elicited limbic afterdischarges in naive and kindled rats without lowering seizure threshold. When fully amygdaloid-kindled rats are electrically stimulated at intertrial stimulation intervals of less than 60 minutes, significant residual inhibition can be demonstrated. The present study examines the effect of three doses of aminophylline (25, 50 and 100 mg/kg) on repeated daily stimulations of fully amygdaloid-kindled rats. After 100 mg/kg aminophylline, the first elicited amygdaloid-kindled seizure afterdischarge was doubled in length compared to saline controls. The second elicited seizure 15 minutes later resulted in status epilepticus and hindlimb extension in the majority of the aminophylline-treated animals with death occurring in 28%. When 25 or 50 mg/kg of aminophylline was given daily for five days before the first of five daily stimulation trials, each separated by 15 minutes, no significant reduction in postictal inhibition was demonstrated compared to saline controls. The 50 mg/kg aminophylline dose consistently and significantly lengthened only the first afterdischarge of each day without affecting the postictal inhibition seen with repeated stimulations. The neural substrate that governs immediate postictal inhibition of amygdaloid-kindled seizures appears to be resistent to modification by aminophylline at low doses. At high doses of aminophylline (100 mg/kg), sustained epileptical activity occurred. The sustained seizure activity seen at the high dose of aminophylline may be secondary to blockade of the processes which normally terminate seizure activity, or it may represent actual inhibition of the immediate postictal inhibitory processes.

Aminophylline Theophylline Adenosine Kindling Grouped-trial stimulation Inhibition Amygdala

IN his major paper on the kindling phenomenon, Goddard *et al.* [13], noted that if the interstimulus or intertrial interval was shorter than 20 minutes during the acquisition of the amygdaloid-kindled seizure, the kindled response would not develop. As noted by Goddard and others, this postictal process can inhibit the development of the kindled seizure if seizures are elicited too closely during the acquisition phase of kindling [13, 17, 19, 22], or it can raise the threshold and/or prevent the expression of a fully kindled seizure [12]. This postictal refractory period is thought to last between 30 and 60 minutes for both the acquisition phase of amygdaloid-kindling [13,17] and the fully kindled phase [11, 12, 14, 18, 23, 24]. By stimulating animals within this relative refractory period, drug effects on this inhibitory process can be studied.

The methylxanthines, aminophylline (85% theophylline and 15% ethylenediamine) and caffeine have previously been shown to increase the clinical severity and length of electrically elicited limbic afterdischarges in rats [3, 4, 8, 10]. This effect was found before, during, and after limbic and cortical kindling [3, 4, 8] and was not found to be related to alteration of seizure threshold [3, 4, 10]. One explanation for these findings is that the methylxanthines interfere with the processes which terminate or inhibit the prolongation of electrical seizure activity [3,4].

Possible cellular mechanisms by which the methylxanthines could modulate the termination of seizures includes inhibition of brain phosphodiesterase activity and/or by blockade of central nervous system adenosine receptors [20]. Adenosine receptor blockade is thought to be the most sensitive mechanism by which the methylxanthines act, although, other possible mechanisms such as translocation of intracellular calcium and inhibition of endogenous benzodiazepine receptor binding have also been discussed [15, 16, 20].

The present study examines the interaction of various doses of aminophylline with the postictal inhibitory processes of kindling. Fully amygdaloid-kindled rats are studied at an intertrial or interstimulation interval that normally results in immediate postictal inhibition. These grouped trials are repeated over five days with and without aminophylline,

[1]Requests for reprints should be addressed to T. E. Albertson, M.D., Ph.D., Department of Internal Medicine, Trailer 1219, University of California, Davis, Medical Center, 2315 Stockton Boulevard, Sacramento, CA 95817.

looking for evidence of a reduction of both the immediate inhibitory processes and the more progressive delayed inhibitory processes which have been suggested with grouped trial stimulations [13, 18, 19, 23, 25].

METHOD

Fourteen male Sprague-Dawley rats, weighing from 300–325 grams, were the subjects. They were housed individually, had ready access to food, and were maintained on a constant 7 a.m. to 7 p.m. light-dark cycle. All manipulations were done starting between 7 and 8 a.m.

Subjects were anesthetized with Chloropent® (3.6 ml/kg) (a chloral hydrate, pentobarbital, magnesium sulfate mixture) IP. The skull was exposed, and holes were drilled with a dental burr to place electrodes. An electrode consisting of a pair of 34-gauge stainless steel wires, twisted tightly together and insulated except at the tips, was lowered into the right amygdala using the coordinates: 1.0 mm posterior to bregma, 4.75 mm lateral of the midline, and 7.5 mm ventral from the surface of the brain (stereotaxic orientation—incisor bar 5 mm above interaural line). Stainless steel screws were placed over the left parietal cortex and the frontal sinus to serve as recording and reference electrodes, respectively. Additional screws were placed to anchor the final electrode assembly to the skull. The amygdaloid, cortical, and reference electrodes were connected by insulated wires to male Amphenol® connector pins and inserted into a male Amphenol® connector strip. This assembly was attached to the skull with dental acrylic cement. Animals were allowed at least ten days to recover from the surgical procedures before further manipulations.

For each kindling trial, subjects were placed in a Plexiglas box, 30×30×45 cm in size. The electrodes were connected via a central cable to the stimulating and recording equipment. Electrical stimulation was produced by a Grass S11 isolated stimulator and delivered to the amygdala through constant current outputs. The stimulus consisted of a one second train of 60 Hz biphasic square waves. Each square wave was one millisecond in duration and 400 μA in amplitude. At the termination of the stimulus train, the amygdala and the EEG electrodes were electronically switched to a Grass Model 78D polygraph. Electrical activity from the amygdala and the cortex was recorded until all evidence of seizure activity had ceased.

Two measures of seizure severity were employed. The first was the afterdischarge duration (AD) elicited by the stimulus. The AD was defined to be the period during which three or more spikes, of at least twice the maximal prestimulus amplitude, occurred at a frequency of one per second or faster, in the amygdala and/or cortex. If additional AD occurred within one minute of the termination of the proceeding AD, it was included when determining total AD duration. The second measure employed was an assessment of behavioral seizure severity. A ranking scale, similar to that described by Racine [21] was used in which a score of '0' was assigned for no behavioral response; '1' indicated facial clonus; '2' indicated one plus head nodding or head and neck clonus; '3' indicated two plus forelimb clonus; '4' indicated three plus rearing; and '5' indicated repeated rearing and falling over onto the cage floor.

Ten days after implantation of the electrodes, the subjects began daily kindling trials until at least five stable ADs (less than 15% variability) with seizure ranks of five had been elicited. The animals were then randomized into two groups of seven each. The first group received 100 mg/kg of aminophylline (IP), and the second control group received saline (1 ml/kg) prior to stimulation. Fifteen minutes later, both groups received the previously described suprathreshold amygdaloid stimulation. Animals were scored for elicited seizure rank and AD length. Fifteen minutes after the first stimulation, a second suprathreshold stimulation was delivered. Because of the high incidence of status epilepticus (AD longer than five minutes) and the increased clinical severity (hindlimb extension), diazepam 0.5 mg/kg IP was given to four of seven of the aminophylline treated animals, and no further stimulations occurred.

The seven animals that made up the saline control group were used for the rest of the experiments and received a single daily stimulation for five consecutive days starting the next week (Week 1). Following this control week, a schedule in which five consecutive days of stimulation per week was used. For Weeks 2 and 3, animals were randomly assigned each week to receive either saline (1.0 ml/kg) or aminophylline (25 mg/kg) prior to the first stimulation on each of the five days. The first daily stimulation occurred 15 minutes after the injection. Stimulations were repeated every 15 minutes for a total of five trials each day. During Weeks 4 and 5, the animals were tested each week with saline and aminophylline (50 mg/kg) given before the daily grouped trial stimulations. During the final week (Week 6), all animals received single daily control stimulations without drug pretreatments.

Parametric measures such as AD duration were compared to control using Student's t-test and three-way analysis of variance. The non-parametric rank scores were compared using the Mann-Whitney U-test. Histological verification of electrode placement was performed on all animals.

TABLE 1

EFFECTS OF AMINOPHYLLINE (100 mg/kg) ON REPEATED KINDLED AMYGDALOID STIMULATION

		Stimulation*			
		One		Two	
Treatment	N	AD	R	AD	R
Saline (ml/kg)	7	92(10)	4.7	57(19)	2.6
Aminophylline (100 mg/kg)	7	191(20)‡	5	228(42)‡	3.4†

*First stimulation 15 minutes after injection, and second stimulation 15 minutes after first.

†Four of seven animals developed status epilepticus, and two of the four died in status epilepticus.

R=Seizure rank; AD=afterdischarge duration; (SEM); ‡$p<0.01$ compared to saline.

RESULTS

The effects of various doses of aminophylline pretreatment on repeated amygdaloid-kindled seizures are presented in Table 1 and Figs. 1 and 2. Amygdaloid-kindled animals pretreated with 100 mg/kg aminophylline were 'excited,' but none showed any clinical or EEG activity suggesting seizures prior to the first stimulation. With the second stimulation 15 minutes after the first, 57% of the aminophylline-treated animals developed prolonged sustained afterdischarges (greater than five minutes) and increased se-

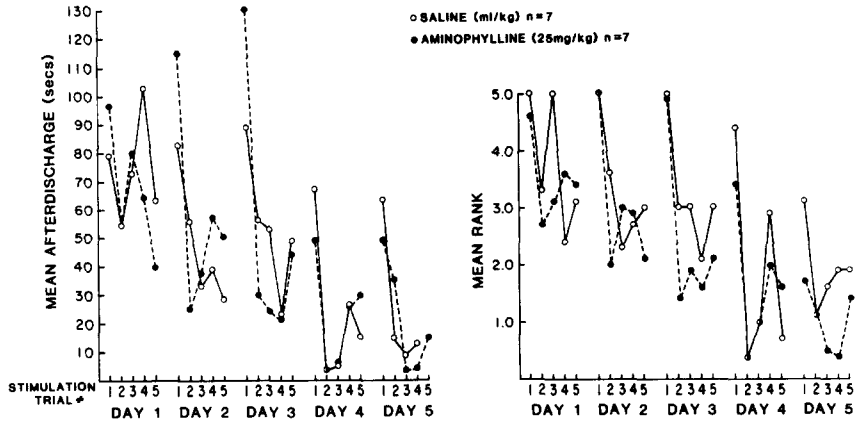

FIG. 1. The effects on elicited afterdischarge duration and seizure rank of daily dosing with 25 mg/kg aminophylline on five days of grouped trial stimulations of amygdaloid-kindled rats are shown.

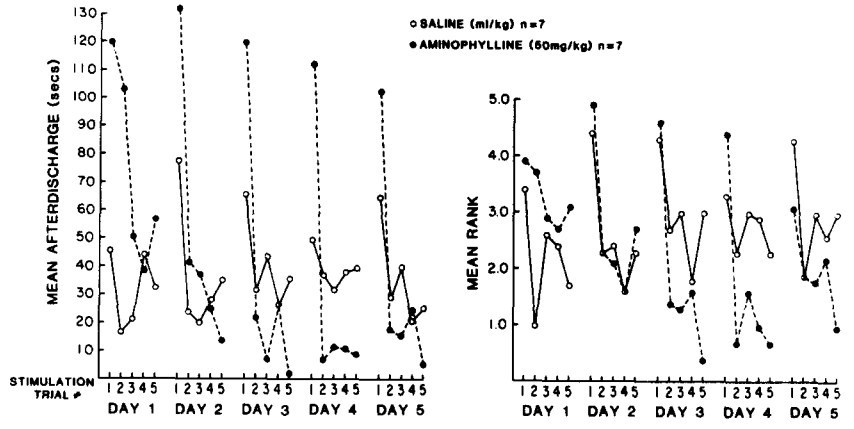

FIG. 2. The effects of daily dosing with 50 mg/kg aminophylline on five days of grouped trial stimulations of amygdaloid-kindled rats are shown.

verity of clinical seizures (including hindlimb extension). A total of 28% of aminophylline-treated animals died in status epilepticus despite attempts to terminate the seizure activity with diazepam (0.5 mg/kg). Animals with ADs greater than five minutes (status epilepticus) were assigned 300 second afterdischarge durations for data analysis purposes. All surviving animals of the 100 mg/kg aminophylline-treated group were discarded from further experiments.

The effect of 25 mg/kg and 50 mg/kg of aminophylline on repeated grouped trial stimulations of amygdaloid-kindled rats is demonstrated in Figs. 1 and 2. Significant ($p \leq 0.05$) day effect on afterdischarge duration, $F(4,48)=15.47$, and trial effect, $F(4,48)=19.40$, were seen at the 25 mg/kg aminophylline dose. No significant drug effect nor interactions were found on three-way ANOVA. Although neither drug nor day effects were seen at the 50 mg/kg aminophylline dose, a significant drug and day interaction was found, $F(4,48)=2.89$. In addition, significant trial effect, $F(4,48)=24.24$, and drug by trial interaction, $F(4,48)=6.95$, were noted with the 50 mg/kg aminophylline dose. The first elicited AD of each day was significantly prolonged after 50 mg/kg aminophylline treatment compared to saline control values.

A comparison between single daily stimulation control Week 1 and Week 6 is shown in Table 2. Significant reduction in both seizure rank and afterdischarge duration was seen on Day 1 of Control Week 6 testing. The differences narrowed by the second day. A suggestion of a residual effect from the previous week of stimulation can also be seen in the reduction in AD and seizure rank scores seen in the saline control group for the 50 mg/kg aminophylline group. This apparent residual effect of the previous week of grouped trial stimulations occurred despite 72 hours without stimulation.

DISCUSSION

In this study, the proconvulsant potential of aminophylline was demonstrated with the prolongation of the first daily elicited AD after pretreatment with doses of 50 and 100 mg/kg of aminophylline. This finding is consistent with previous reports describing the effects of aminophylline in the amygdala, neocortex and hippocampus before and after kindling [2–4, 8, 10]. Complementary reports have pointed to the anticonvulsant properites of adenosine agoinsts in these models [4, 5, 10], further suggesting an important role for adenosine in the modulation of seizure termination. Al-

TABLE 2

CONTROL WEEK ELICITED SEIZURE RANKS AND AFTERDISCHARGE DURATIONS AFTER SINGLE DAILY STIMULATIONS OF FULLY AMYGDALOID-KINDLED RATS

	Day									
	1		2		3		4		5	
Week	AD	R	AD	R	AD	R	AD	R	AD	R
One	91 (9)	5.0	89 (8)	5.0	90 (9)	5.0	89(9)	5.0	77(11)	4.3
Six	66(13)*	2.6*	75(10)	3.9	89(13)	5.0	83(6)	5.0	103 (8)	5.0

N=7: *$p<0.05$ compared to Week One; (SEM); AD=afterdischarge duration (sec); R=seizure rank.

though the highest dose of aminophylline used in this study is perhaps enough to inhibit phosphodiesterase activity, effect translocation of intracellular calcium and inhibit benzodiazepine receptor binding [15, 16, 20], a large amount of evidence points to a mechanism of action of the methylxanthines through blockade of the adenosine receptor [20].

Some investigators have reported lowered pentylenetetrazol and maximal electroshock seizure thresholds after high doses of aminophylline (150 mg/kg) [26]. This laboratory and others have previously seen no significant change after methylxanthines in the electrical thresholds for amygdaloid-kindled seizures or perforant path-dentate monosynaptic evoked potentials before or after kindling [2–4, 10]. These findings provide evidence for a lack of general lowering of seizure thresholds as the major mechanisms for the effect of methylxanthines on kindled seizures.

With the highest dose of aminophylline used in this study (100 mg/kg), prolonged elicited seizures occurred after the second stimulation with most animals developing status epilepticus (AD greater than five minutes). Many of the animals did not survive the episode of status epilepticus despite acute anticonvulsant intervention. Previous studies have shown that amygdaloid-kindled rats can tolerate doses of 100–150 mg/kg of theophylline or aminophylline prior to elicited kindled seizures with a resulting increase in AD length but without the development of status epilepticus and death being reported in a high percentage of treated animals [4,8]. Spontaneous seizures have been reported with aminophylline in nonkindled rats at doses of 200 mg/kg or greater [9,26] and 150 mg/kg in amygdaloid-kindled rats [4]. Together, these data would appear to point to the fact that high dose aminophylline is capable of blocking the postictal inhibition seen after an elicited amygdaloid-kindled seizure. An actual summation or addition of proconvulsant effects between the aminophylline and the previous stimulation appears to occur, allowing the development of prolonged AD and status epilepticus with the second elicited seizure. Further studies with other convulsants such as pentylenetetrazol and bicuculine, both known to increase AD length [6,7], are needed to determine if the findings on postical inhibition with aminophylline are specific or are a general finding with convulsants. Further pharmacokinetic studies are needed to eliminate drug level fluxes as a contributing factor and to better understand this as a potential model of human status epilepticus.

It is of interest that the lower doses of aminophylline (25 and 50 mg/kg) tested in this study tended to result in prolongation of the initial daily elicited AD consistent with previous reports [4, 8, 10]. The 50 mg/kg aminophylline dose consistently lengthened the initial elicited AD of each day without lengthening the following daily elicited ADs of the group trial stimulations. Thus, the postictal inhibition seen with group trial stimulations was not reversed despite an exposure to a dose of aminophylline which can prolong initial elicited ADs in amygdaloid-kindled rats. Significant trial effects were seen with both aminophylline doses confirming the presence of postictal inhibition. In addition, the significant drug by day and drug by trial interactions seen with the 50 mg/kg aminophylline dose points towards a complicated interaction of this dose of aminophylline with the stimulation paradigm and a possible increase in postictal inhibition. The loss of stable seizure response and increased variability of AD duration seen on the first two days of single seizure testing during Control Week 6 further suggests that daily group trial stimulation paradigms initiate delayed inhibitory processes which further complicates drug effect interpretations [1].

It would appear that the ability of the methylxanthine aminophylline to prolong elicited amygdaloid-kindled ADs (perhaps by interfering with seizure termination) can occur at doses that do not result in significant reduction in the postictal inhibition seen with group trials or the recurrent collateral inhibition seen with perforant path to dentate gyrus evoked responses [2]. It also appears that higher doses of aminophylline can prolong elicited amygdaloid-kindled ADs and also block post-seizure inhibition allowing sustained elicited ADs. This duality of findings probably reflects various cellular dose dependent effects of the methylxanthines.

ACKNOWLEDGEMENTS

The technical assistance of W. F. Walby and J. Streisand and the typing of S. Cox are gratefully acknowledged. Supported in part by Biomedical Research Support Grant, U. C. Davis, 2507RR-05684-16 and by the Health Sciences Neurotoxicology Unit.

REFERENCES

1. Albertson, T. E. Amygdaloid-kindled postical inhibition: effect of intertrial intervals on repeated days. *Exp Neurol* **92:** 197–206, 1986.
2. Albertson, T. E. and R. M. Joy. Modification of excitation and inhibition evoked in dentate gyrus by perforant path stimulation: Effects of aminophylline and kindling. *Pharmacol Biochem Behav* **24:** 85–91, 1986.
3. Albertson, T. E., R. M. Joy and L. G. Stark. Caffeine modification of kindled amygdaloid seizures. *Pharmacol Biochem Behav* **19:** 339–343, 1983.
4. Albertson, T. E., L. G. Stark, R. M. Joy and J. F. Bowyer. Aminophylline and kindled seizures. *Exp Neurol* **81:** 703–713, 1983.
5. Barraco, R. A., T. H. Swanson, J. W. Phillis and R. F. Berman. Anticonvulsant effects of adenosine analogues on amygdaloid-kindled seizures in rats. *Neurosci Lett* **46:** 317–322, 1984.
6. Bowyer, J. F. and T. E. Albertson. The effects of pentylenetetrazol, bicuculline and strychnine on the development of kindled seizures. *Neuropharmacology* **21:** 985–990, 1982.
7. Bowyer, J. F., W. D. Winters and T. E. Albertson. Pentylenetetrazol augmentation of developing kindled seizures. *Neuropharmacology* **20:** 1225–1227, 1981.
8. Cain, D. P. Kindling: Recent studies and new directions. In: *Kindling 2*, edited by J. A. Wada. New York: Raven Press, 1981, pp. 49–66.
9. Chu, N.-S. Caffeine- and aminophylline-induced seizures. *Epilepsia* **22:** 85–94, 1981.
10. Dragunon, M. and G. V. Goddard. Adenosine modulation of amygdala kindling. *Exp Neurol* **84:** 654–665, 1984.
11. Engel, J. and R. F. Ackermann. Interictal EEG spikes correlate with decreased, rather than increased, epilepto-genicity in amygdaloid kindled rats. *Brain Res* **190:** 543–548, 1980.
12. Freeman, F. G. and M. F. Jarvis. The effect of interstimulation interval on the assessment and stability of kindled seizure thresholds. *Brain Res Bull* **7:** 629–633, 1981.
13. Goddard, G. V., D. C. McIntyre and C. K. Leech. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* **25:** 295–330, 1969.
14. Jarvis, M. F. and F. G. Freeman. The effects of naloxone and interstimulation interval on postictal depression in kindled seizures. *Brain Res* **288:** 235–241, 1983.
15. Marangos, P. J., A. M. Martino, S. M. Paul and P. S. Kolnick. The benzodiazepines and inosine antagonize caffeine-induced seizures. *Psychopharmacology (Berlin)* **72:** 269–273, 1981.
16. Marangos, P. J., S. M. Paul, A. M. Parma, F. K. Goodwin, P. Syapin and P. Skolnick. Purinergic inhibition of diazepam binding to rat brain (in vitro). *Life Sci* **24:** 851–858, 1979.
17. Moshe, S. L., B. J. Albala, R. F. Ackermann and J. Engel. Increased seizure susceptibility of the immature brain. *Dev Brain Res* **7:** 81–85, 1983.
18. Mucha, R. F. and P. J. Pinel. Post seizure inhibition of kindled seizures. *Exp Neurol* **54:** 266–282, 1977.
19. Peterson, S. L., T. E. Albertson and L. G. Stark. Intertrial intervals and kindled seizures. *Exp Neurol* **71:** 144–153, 1981.
20. Phillis, J. W. and P. H. Wu. The role of adenosine and its nucleotides in central synaptic transmission. *Prog Neurobiol* **16:** 187–239, 1981.
21. Racine, R. J. Modification of seizure activity by electrical stimulation: II motor seizure. *Electroencephalogr Clin Neurophysiol* **32:** 281–294, 1972.
22. Racine, R. J., W. M. Burnham, J. G. Gartner and D. Levitan. Rates of motor seizure development in rats subjected to electrical brain stimulation: Strain and interstimulation interval effects. *Electroencephalogr Clin Neurophysiol* **35:** 553–556, 1973.
23. Sainsbury, R. S., B. H. Bland and D. H. Buchan. Electrically induced seisure activity in the hippocampus: Time course for post seizure inhibition of subsequent kindled seizures. *Behav Biol* **22:** 479–488, 1978.
24. Sato, M. and T. Moriwake. Postictal seizure inhibition in amygdaloid-kindled cats. *Epilepsia* **25:** 545–550, 1984.
25. Shao, J. and E. S. Valenstein. Long-term inhibition of kindled seizures by brain stimulation. *Exp Neurol* **76:** 376–392, 1982.
26. Walker, J. E. Effect of aminophylline on seizure thresholds and brain regional cyclic nucleotides in the rat. *Exp Neurol* **74:** 299–304, 1981.