Pharmacology Biochemistry & Behavior, Vol. 24, pp. 85–91, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Modification of Excitation and Inhibition Evoked in Dentate Gyrus by Perforant Path Stimulation: Effects of Aminophylline and Kindling[1]

T. E. ALBERTSON[2] AND R. M. JOY

Health Sciences Neurotoxicology Unit, Departments of Internal Medicine, School of Medicine and Pharmacology and Toxicology, School of Veterinary Medicine University of California, Davis, CA 95616

Received 19 February 1985

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**(1) 85–91, 1986.—Rats were implanted with chronic electrodes to stimulate the perforant path and record the elicited monosynaptic evoked potentials from the dentate gyrus of the hippocampal formation. Dentate responses were examined in awake and anesthetized animals after exposure to saline and aminophylline (100 mg/kg, IP). In the awake animal, aminophylline treatment did not significantly alter the threshold or elicited amplitude of either the excitatory post-synaptic potential (EPSP) or the population spike (PS). Aminophylline pretreatment markedly enhanced the length and severity of elicited seizures from hippocampal (dentate gyrus) or perforant pathway stimulation. After daily perforant pathway stimulations which established "kindled" seizures, aminophylline significantly increased only the amplitude of the evoked PS in awake animals. In animals anesthetized with chloropent, aminophylline increased significantly before kindling the amplitude of both the EPSP and PS without effecting thresholds for each. After perforant pathway kindling, only the PS amplitude was increased significantly by aminophylline. Inhibition, thought to be from GABA-mediated recurrent collaterals, was found to be increased rather than decreased by kindling. Further, aminophylline treatment did not result in reduction of this inhibition before or after kindling. These data suggest that at this dose of aminophylline neither enhanced transmitter release at this synapse as measured by the amplitude of the EPSP, nor reduced recurrent collateral inhibition significantly contributed to the prolongation of elicited seizure afterdischarge. The increase in PS amplitude reflecting an increased number of granule cells excited to discharge with perforant path stimulation after aminophylline was noted in awake animals but was greatest in the anesthetized animals. Although the number of granule cells excited to discharge was increased by aminophylline, the small increase in amplitude seen compared to the effects of other neurotoxins on this synapse makes this an unlikely explanation for the profound increased seizure response seen after aminophylline.

Aminophylline Theophylline Adenosine Evoked potentials Recurrent collateral inhibition
Kindling Perforant path Dentate gyrus

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, 10, 16]. This effect was found before, during and after limbic and cortical kindling [3, 4, 10, 11] and was not found to be related to alteration of seizure threshold [3,4]. One explanation for these findings is that the methylxanthines interfere with the processes which terminate 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 [36]. 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 [32, 33, 36].

The limbic system is well suited for further electrophysiological studies on the mechanism of action of the methylxanthines. The hippocampal formation has a relatively simple, well laminated structure with well defined afferents and efferents [11, 20, 45]. The neurotransmitters acting at the various synapses have been tentatively identified in most cases [45]. A consistency of structure and extensive

[1]This work supported in part by BRS Grant No. 2SO7RRO5684-16 and a Faculty Research Grant.

[2]Requests for reprints should be addressed to T. E. Albertson, M. D., Ph.D., Department of Internal Medicine, Division Emergency Medicine and Clinical Toxicology, University of California, Davis Medical Center, 2315 Stockton Blvd., Sacramento, CA 95817.

lamination yields evoked field responses to activation of various afferent pathways that are large in amplitude and easy to measure. Extensive analysis of these potentials has led to acceptance of the origin of certain of the evoked response components [5, 6, 30, 35]. The dentate field response to stimulation of the perforant path is monosynaptic and can be readily separated into components produced by the synchronously elicited population excitatory postsynaptic potentials (EPSP) and the synchronously elicited cell discharges or population spikes (PS) [5,6]. Many of the synapses within the hippocampal formation exhibit strong neuroplastic reactions to repetitive stimuli. They have been shown to exhibit short-term potentiation, long-term potentiation and related phenomena which are potentially important mechanisms involved in kindling [8, 9, 14, 15, 30, 31, 39, 40].

The perforant path to dentate evoked potential has been used to evaluate the mechanism of action of the chlorinated hydrocarbon lindane, a drug which has been shown to increase the rate of limbic kindling but to have no effect on the fully kindled seizure [21]. Other laboratories have also utilized this monosynaptic limbic evoked potential to evaluate several other drugs and their mechanisms of action including their effects on recurrent collateral inhibition [1, 18, 46].

In this paper we report an analysis of the effects of the methylxanthine, aminophylline, on responses evoked in the dentate gyrus to perforant path stimulation before and after perforant path kindling.

METHOD

Surgical Preparation

Four male Sprague-Dawley rats, 250–350 g, were anesthetized with 3.6 ml/kg of Chloropent® (Fort Dodge Laboratories; each milliliter contains 42.5 mg chloral hydrate, 21.2 mg $MgSO_4$, 8.9 mg pentobarbital and 14.3% ethyl alcohol) and placed into a stereotaxic device (incisor bar 5 mm above the intraaural line). The skull was exposed, and 2 mm diameter holes were drilled to place electrodes. The stimulating electrodes were made from twisted pairs of 34 gauge stainless steel wires insulated except at the tips which were 1 mm apart. The electrode was placed into the right perforant path at the medial entorhinal cortex (near the angular bundle) using coordinates of 7.0 mm posterior to bregma, 4.1 mm lateral to midline and 3.2 mm below the cortical surface. A recording electrode of similar design was inserted into the dorsal blade of the right dentate gyrus (2.0 mm posterior to bregma, 1.0 mm lateral to the midline and 3.5 mm below the cortical surface). A stainless steel screw was placed over the frontal sinus to serve as ground. The electrodes were inserted into a 5-pin Amphenol® connector which was attached to the skull with dental acrylic cement. Two weeks were allowed for recovery.

Recording and Stimulation

Stimuli were square-wave pulses formed by a WPI Model 830 stimulator delivered through WPI Model 850 isolation units. Pulses were 0.1 msec in duration and varied in amplitude from 3 to 50 volts. In most cases, pulse pairs (interstimulus interval=30 msec) were employed at a repetition frequency of 0.2 Hz.

Dentate responses were amplified using Grass Model 7P511 amplifiers with low and high frequency cutoffs at 3 Hz and 10 kHz. Responses were observed on a Tektronix 565 and a Nicolet 3091 digital storage oscilloscope. The responses were also recorded on a Hewlett-Packard Model 3521 FM tape recorder. For purpose of data analysis, groups of ten responses were summed at intervals on a Mnemetron Computer of Average Transients, and the resultant was plotted out on a Grass Model 78B polygraph.

TABLE 1

EFFECT OF AMINOPHYLLINE ON DENTATE GYRUS RESPONSE TO PERFORANT PATH STIMULATION IN AWAKE CHRONICALLY IMPLANTED RATS

State	Measure		Saline	Aminophylline
Prekindled	Amplitude	EPSP	97.5 ± 5.9‡	108.3 ± 7.8
		PS	83.1 ± 5.6*	109.5 ± 5.6
	Threshold	EPSP	97 ± 1.2	99.3 ± 2.8
		PS	98 ± 1.4	105 ± 3.2
Kindled§	Amplitude	EPSP	96.5 ± 3.6	105.8 ± 4.8
		PS	83.5 ± 4.6†	111.1 ± 3.1†
	Threshold	EPSP	101 ± 0.6	102 ± 1.2
		PS	99 ± 1	98 ± 0.5

*$p \leq 0.05$, †$p \leq 0.01$.
‡Values in percent of control trial (mean ± SEM).
§Perforant path kindling.

Procedure—Evoked Potentials

Each subject was placed in a Plexiglas box (30×30×45) twice weekly and stimulated until stable evoked responses were obtained. This usually took two to three weeks. Each animal was then randomly tested weekly in one of three paradigms: (1) an awake control period followed by an awake saline (1 ml/kg) control period 15 minutes after injection and an aminophylline (100 mg/kg) test period 15 minutes after injection; (2) an awake control period followed by an anesthetized control period (3.6 ml/kg IP Chloropent®) followed by a saline (1 ml/kg) test period 15 minutes after injection; and (3) an awake control period followed by an anesthetized control period followed by an aminophylline (100 mg/kg) test period.

In each situation, the subject was given 15 minutes to acclimatize, then awake thresholds for evoking the EPSP and PS were determined. The "low" stimulus intensity was adjusted to generate a pure EPSP uncontaminated by a PS at approximately 70–80% of the PS threshold. The "high" stimulus intensity was adjusted to produce a PS amplitude 70–80% the maximal attainable. Five averages of ten responses each were then gathered. Next, pulse pairs of equal voltage and duration (0.1 msec) were presented with interpulse intervals ranging from 10–200 msec. Averages of ten responses at each interval tested were formed and plotted for later analysis. This procedure was repeated for each of the three weekly paradigms. During evoked potential testing, anesthesia was maintained at depths which abolished withdrawal responses to toe pinch by administering additional 0.5 ml/kg doses of Chloropent®, IP, at 60-minute intervals. Body temperature was kept at 38°±1°C with a constant temperature heating pad and wraps.

Data Analysis—Evoked Potentials

The analysis of waveforms followed closely that de-

TABLE 2

EFFECT OF AMINOPHYLLINE ON DENTATE GYRUS RESPONSE TO PERFORANT PATH STIMULATION IN ANESTHETIZED CHRONICALLY IMPLANTED RATS

State	Measure		Anesthetized	Saline	Aminophylline
Prekindled	Amplitude	EPSP	92 ± 9.2§	102.6 ± 1.5¶	121.9 ± 16.4*¶
		PS	67.9 ± 7.6‡	98.5 ± 3.3	140.7 ± 22.0†
	Threshold	EPSP	100.2 ± 2.2	101.5 ± 2.5	103.5 ± 3.7
		PS	110.5 ± 7.4	105.9 ± 4.8	98.5 ± 1.1
Kindled#	Amplitude	EPSP	92.1 ± 6.0*	92.7 ± 6.9*	103.5 ± 6.0
		PS	55.7 ± 3.8‡	111.7 ± 7.8	127.2 ± 6.0‡
	Threshold	EPSP	101.4 ± 1.3	99.2 ± 0.7	101.7 ± 1.3
		PS	107 ± 3.5	107.9 ± 4.8	103.4 ± 2.3

*$p \leq 0.05$, †$p \leq 0.01$, ‡$p \leq 0.001$.
§Values (mean ± SEM) in percent of awake controls.
¶Saline and aminophylline are in percent of anesthetized control values (mean ± SEM).
#Perforant path kindling.

scribed by Bliss and Lomo [8] and Douglas and Goddard [15]. The EPSP was defined on the basis of latency and on its ability to follow stimulation frequencies above 50 Hz [15]. This latter test was done at the end of the experiment. The amplitude of the EPSP was determined from records where a pure EPSP uncontaminated by a PS was observed or else on the basis of its slope in the period before the PS began. The PS was defined by its waveform and latency and by its response to a preceeding stimulus. Preceeding stimuli below threshold for evoking a PS typically potentiated the response to the second stimulus while preceeding stimuli strong enough to evoke a PS typically abolished the PS to the second stimuli by activating recurrent inhibition.

During each of the various periods tested in each paradigm, the following measurements were obtained: (1) EPSP threshold; (2) PS threshold; (3) PS amplitude (single stimulus); and (4) PS amplitudes (paired stimuli). When pairs of stimuli were used, the amplitude of both the first (PS1) and the second (PS2) population spike was determined. The ratio (PS2)/(PS1) was derived to estimate the extent of recurrent collateral inhibition produced by PS1 during the paired-pulse studies.

Interaction Between Kindling and Aminophylline Administration

The four subjects chronically implanted with stimulation and recording electrodes from the above study and two additional similarly implanted animals which had not undergone evoked potential testing were stimulated twice weekly 15 minutes after saline (1 ml/kg) or aminophylline (100 mg/kg) through either the perforant path electrode or the dentate electrode using a modified Grass S-44 stimulator that delivered 400 μA stimulus of 1-sec train of 60 Hz biphasic square waves, each 1 msec in duration. Electrical activity from the dentate and the perforant pathway were recorded until all evidence of seizure activity had ceased. Observation of seizure rank (0–5 scale) was made for each trial. After these four trials, animals were not stimulated for three days, then daily perforant path stimulations with the same stimulation parameters were done until stable, fully kindled seizures developed (seizure rank 5). When kindling was complete, subjects were evaluated for the effects of aminophylline on evoked responses in a manner similar to that previously described. After this, the animals were again subjected randomly to twice-weekly seizure stimulation of either the perforant path or dentate gyrus 15 minutes after either saline (1 ml/kg) or aminophylline (100 mg/kg) exposure.

TABLE 3

RECURRENT INHIBITION STUDY OF POPULATION SPIKES IN AWAKE CHRONICALLY IMPLANTED RATS

State	Saline	Aminophylline
Prekindled	88.3 ± 19.1†‡	108.0 ± 23.0
Kindled	85.4 ± 5.1*	104.2 ± 4.0

*$p \leq 0.05$.
†Mean ± SEM.
‡Values in mean percent of awake control measured as time to 50% recovery of PS2 as compared to PS1 expressed as percent of awake control. Awake prekindled control time=23 ± 6.6 msec (N=4) and awake kindled control time=42.4 ± 2.0 msec (N=5).

Statistical Evaluation

Evoked response comparisons between control, saline and aminophylline administration were made between groups using paired t-test. The effects of aminophylline on perforant path or dentate elicited seizure afterdischarge durations were compared using the Student's t-test. All nonparametric measures, including rank scores and transformation of rank scores, were made using the Mann-Whitney U-test or a signed ranks test. Histological verification of electrode placement revealed no major variations.

RESULTS

Perforant Path-Dentate Gyrus Evoked Potentials

Previous work by this laboratory and others [2, 5, 6, 12, 21, 34, 39, 46] have described and illustrated the various components of the perforant path-dentate gyrus evoked potentials. The effects of aminophylline on the dentate gyrus evoked potentials after perforant path stimulation in awake

TABLE 4

RECURRENT INHIBITION STUDY OF POPULATION SPIKES IN CHRONICALLY IMPLANTED RATS

State	Awake	Anesthetized	Saline (%)¶	Aminophylline (%)¶
Prekindled	26.5 ± 5.8‡§	60 ± 9.9*	50.3 ± 9.6 (83.8)*	—
(N=4)	27.7 ± 5.4	51.5 ± 2.5*	—	51 ± 5.4 (99)
Kindled	44 ± 6.6	54.6 ± 8	55.2 ± 6.9 (101.3)	—
(N=5)	45 ± 3.4†	50 ± 6.3	—	63 ± 5.7 (126)

*$p \leq 0.05$ compared control same state awake.
†$p \leq 0.05$ compared to prekindled.
‡Mean ± SEM in msec.
§The paired pulse interval at which the second elicited population spike (PS2) amplitude is 50% of the first population spike amplitude (PS1).
¶Percent of anesthetized control.

TABLE 5

PERFORANT PATH KINDLING ACQUISITION

N	Mean Stimulations to First Stage 5 Seizure	Mean Seconds of Afterdischarge Duration of First Stage 5 Seizure	Total Seconds of Accrued Afterdischarge to First Stage 5 Seizure
5	9.2 ± 1.1*	89 ± 20.2	455 ± 41

*Mean ± SEM.

TABLE 6

EFFECTS OF AMINOPHYLLINE AND KINDLING ON HIPPOCAMPAL OR PERFORANT PATHWAY STIMULATION

State	Stimulation	Drug	Afterdischarge (sec)	Rank	% Status
Prekindled	Hippocampus (Dentate)	S	24	0.2	0
		A	200*	5*	84
	Perforant Path	S	34	0.8	0
		A	176*	4*	67
Kindled¶	Hippocampus (Dentate)	S	71†	3.2†	0
		A	190*	5	100
	Perforant Path	S	69†	3.8†	0
		A	127*	4.2	40

*$p \leq 0.05$ compared to saline.
†$p \leq 0.05$ compared to prekindled.
‡S=saline (ml/kg); A=100 mg/kg aminophylline.
§Percent of animals displaying prolonged (greater than 200 sec) severe seizures. Animals in status epilepticus were assigned 200 sec afterdischarge durations.
¶Perforant path kindling.

animals chronically implanted with electrodes before and after perforant path kindling is demonstrated in Table 1. Small increases in the amplitude of the PS and EPSP were seen after 100 mg/kg aminophylline before and after kindling. Only the kindled PS amplitude increase reached statistical significance ($p<0.05$). No changes in EPSP or PS thresholds were noted in the awake animals (Table 1). Table 2 demonstrates that with Chloropent® anesthesia, the PS amplitude was significantly decreased both before and after kindling. The reduction in EPSP amplitude with anesthesia was small and less than the reduction in PS amplitude. With anesthesia, no significant changes in thresholds were also noted after aminophylline (Table 2). Aminophylline significantly increased the PS amplitude in anesthetized subjects before and after kindling (Table 2). The prekindled EPSP amplitude in anesthetized subjects was also significantly increased (Table 2). No consistent change in thresholds was noted with aminophylline before or after kindling. No differences were noted in any measures between the three animals tested before and after kindling compared to the two animals that were kindled without prekindled evoked potential determinations.

Recurrent Inhibition

The rate of recovery of PS2 during paired stimulus presentation was used to provide a measure of recurrent collateral inhibitory strength. In the awake animal, no significant effect of aminophylline was seen on recurrent inhibition measurements (Table 3). A marked increase in control time to reach 50 percent recovery of PS2 was noted after kindling (23±6.6 vs. 42.4±2.0; mean±SEM msec) in the awake animal. The influence of PS1 on PS2 was significant and greatly extended in time by anesthesia before kindling (Table 4). After kindling, anesthesia further increased the 50 percent recovery time of PS2 only an additional 5–10 msec (Table 4). In the anesthetized subject, recurrent inhibition was not significantly changed by aminophylline before or after kindling (Table 4).

Interaction Between Kindling and Aminophylline Administration

The various acquisition parameters of perforant path kindling are shown in Table 5. Aminophylline pretreatment

markedly increased the length and severity of seizures elicited from both hippocampal and perforant path stimulation (Table 6). Hippocampal stimulation with aminophylline pretreatment resulted in the greatest augmentation both in the prekindled and kindled state. A higher percentage of animals went into status epilepticus (afterdischarge duration greater than 200 sec and severe tonic/clonic seizures) with aminophylline after hippocampal stimulation than compared to perforant path stimulation (Table 6). All animals in status epilepticus were treated with 1 mg/kg IP diazepam to terminate the seizure after 200 sec. Despite this treatment, one of the prekindled animals stimulated in the hippocampus died in status epilepticus. Kindling did not further change the response to aminophylline after either hippocampal or perforant path stimulation (Table 6).

DISCUSSION

In interpreting the field potentials from the dentate gyrus, Andersen *et al.* [6] have provided evidence to suggest that the amplitude of the population spike (PS) reflects the number and synchrony of granule cell discharges. Interpretation of the excitatory post-synaptic potential (EPSP) is more complex [1, 5, 6, 8, 9, 14, 15, 24, 30, 35, 38, 40, 46] with an increase in amplitude thought to be due to either: (1) an increase in number of perforant path fibers activated by the stimulus; (2) an increase in the efficacy of the synaptic process through increased transmitter release and/or enhanced receptor properties; and (3) a decrease in the background level of tonic afferent input [8]. A reduction in amplitude of the EPSP may represent opposite influences from those outlined above, such as a decrease in membrane resistances or cellular depolarizations. The data presented in this paper suggests that synaptic activation via perforant path afferents (EPSP amplitude) was not consistently enhanced by aminophylline treatment. Only the prekindled anesthetized state showed a significant, though small, increase in EPSP amplitude with large variability after aminophylline.

A small but consistent increase in granule cell excitability to perforant path input was demonstrated by an increase in PS amplitude after aminophylline before and after kindling. The magnitude of this increase is relatively small compared to other proconvulsant agents such as the chlorinated hydrocarbon, lindane, which has been extensively studied in this model [21,22]. The cause of the increase in granule cell excitability after aminophylline can only be inferred from previous studies. Although the dose of aminophylline used in this study is high enough to potentially inhibit phosphodiesterase activity, effect translocation of intracellular calcium and inhibit benzodiazepine receptor binding [32, 33, 36], a great deal of evidence points to a mechanism of action through blockade of the adenosine receptor [36]. The A_1 adenosine receptor site densities are greater in the dorsal versus ventral hippocampus [25,41] and correlate well with the *in vitro* modulatory action by adenosine of evoked potentials in the hippocampal slice preparation [17, 26, 27, 28, 29, 42]. The methylxanthine theophylline antagonizes the depressive effect of adenosine on neural afterdischarges generated *in vitro* from hippocampal slices in the presence of penicillin or low extracellular calcium concentrations [28]. Further, antidromic stimulation studies in this same preparation [28] have pointed to a post-synaptic site of action of adenosine in regulating repetitive discharges which are characteristic of epileptiform activity. In an *in vivo* study, Dolphin [12] has shown that the long-term potentiation elicited after a brief high frequency stimulation of the perforant path to dentate evoked potential can be inhibited by local infusions near the granule cells of the adenosine agonist, 2-chloroadenosine, while short-term potentiation was still produced. Dolphin and Archer [13] have shown that 2-chloroadenosine also inhibited while theophylline enhanced the K^+-induced release at [^{3}H] glutamate but not [^{3}H] GABA from slices of rat dentate gyrus. Together, this evidence points to the location of adenosine and aminophylline action to most likely be post-synaptic, for example on the granule cells, although presynaptic contributions have not been eliminated [13, 37, 43].

Although some previous workers have found lowered pentylenetetrazol and maximal electroshock seizure thresholds after high dose aminophylline [47], this laboratory and others have previously reported no significant change in the electrical thresholds for amygdaloid kindled seizures after methylxanthines [3, 4, 16]. The present study demonstrated a lack of effect of aminophylline on the electrical threshold needed to elicit an EPSP or PS in the perforant path-dentate monosynaptic evoked potential before and after kindling. This finding is further evidence for the lack of general lowering of thresholds as a major mechanism for the effect of methylxanthines on seizures.

Previous studies have utilized the presumed GABA mediated recurrent inhibition on granule cell PS amplitudes in response to a second perforant path stimulation to determine drug effects on *in vivo* inhibitory function [1, 18, 21, 22, 44, 46]. A reduction of recurrent collateral granule cell inhibition has been reported after trimethyltin, bicuculline and local kainic acid exposure [18, 44, 46]. An increase in inhibition has been seen with diazepam and valproic acid [1,46], while lindane [21,22] and now aminophylline have failed to alter this inhibition. The present study with perforant path kindling is consistent with the previous reports [2,46] in which both amygdala and dentate kindling increased rather than decreased perforant path-dentate recurrent inhibition. Although Winson [48] utilized Chloropent® anesthesia in studying the effects of median raphe prestimulation on perforant path-dentate recurrent inhibition, he failed to directly comment on its effect on inhibition. In this study, Chloropent® significantly increased recurrent inhibition and reduced initial PS amplitude.

Previous studies using slightly different electrode placements and stimulation parameters have reported that 48% of animals were kindled after fourteen days of daily perforant path stimulations in one study [34], and in another a mean of nineteen stimulations were needed to kindle the animals [23]. The 9.2 daily perforant-path stimulations needed to kindle in this study are less than previous reports. Beyond parametric differences, the previous exposures to aminophylline and episodes of status epilepticus before daily kindling events started may have contributed to the reduced number of stimulations needed to kindle in this study.

Although formal I/O studies were not done before and after kindling in this study, no change in EPSP thresholds or amplitudes were noted. This finding is similar to previous reports with monosynaptic evoked potentials from lateral enterohinal cortex to dentate granule cells after kindling [19]. Some investigators have reported increases on EPSP amplitudes with hippocampal kindling [2]. Maru *et al.* [34] have reported a transient increase in evoked perforant path-dentate EPSP amplitude and a variable PS effect with perforant-path kindling, while Tuff *et al.* [46] failed to show a change in either PS or EPSP amplitude in the same perfo-

rant path-dentate evoked potential after either amygdaloid or dentate kindling.

Profound prolongation of the elicited afterdischarges from either perforant path or dentate stimulation with the adenosine antagonist aminophylline is consistent with previous reports regarding its effect in the amygdala and neocortex before and after kindling [3, 4, 10, 16]. Complementary reports have pointed to the anticonvulsant properties of adenosine agonists in these models [4, 7, 16], further suggesting an important role for adenosine in the modulation of seizure termination. Further experiments with lower doses of aminophylline and other adenosine antagonists (e.g., caffeine) and agonists (e.g., 2-chloroadenosine) are needed to better understand the exact role of adenosine modulation in elicited perforant path or dentate afterdischarges and evoked potentials.

Together this study fails to demonstrate a generalized reduction of limbic recurrent inhibition to explain the effect of aminophylline on prolonging limbic seizures before and after kindling. Similarly, granule cell EPSP amplitude and threshold are not obviously involved. The small increases seen in PS amplitude with aminophylline before and after kindling primarily in the anesthesized state seems unlikely to account for the lack of termination of seizures seen with aminophylline. Further, *in vivo* and *in vitro* studies to better understand the mechanisms involved in terminating seizures and the interaction of kindling and aminophylline are needed.

REFERENCES

1. Adamec, R. E., B. McNaughton, R. Racine and K. E. Livingston. Effects of diazepam on hippocampal excitability in the rat: Action in the dentate area. *Epilepsia* **22:** 205–215, 1981.
2. Adamec, R. E. and C. Stark-Adamec. Partial kindling and emotional bias in the cat: Lasting after effects of partial kindling of ventral hippocampus. *Behav Neural Biol* **38:** 223–239, 1983.
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. Andersen, P., B. Holmquist and P. E. Voorhoeve. Entorhinal activation of dentate granule cells. *Acta Physiol Scand* **66:** 448–460, 1966.
6. Andersen, P., T. V. P. Bliss and K. K. Skrede. Unit analysis of hippocampal population spikes. *Exp Brain Res* **13:** 208–221, 1971.
7. 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.
8. Bliss, T. V. P. and T. Lomo. Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *J Physiol (Lond)* **232:** 331–356, 1973.
9. Bliss, T. V. P. and A. R. Gardner-Medwin. Long-lasting potentiation of synaptic transmission in the dentate area of the unanesthetized rabbit following stimulation of the perforant path. *J Physiol (Lond)* **232:** 357–374, 1973.
10. 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.
11. Cowan, W. M., B. B. Stanfield and K. Kishi. The development of the dentate gyrus. In: *Current Topics in Development Biology, Vol 2*, edited by R. K. Hunt. New York: Academic Press, 1980, pp. 103–157.
12. Dolphin, A. C. The adenosine agonist 2-chloroadenosine inhibits the induction of long-term potentiation of the perforant path. *Neurosci Lett* **39:** 83–89, 1983.
13. Dolphin, A. C. and E. R. Archer. An adenosine agonist inhibits and a cyclic AMP analogue enhances the release of glutamate but not GABA from slices of rat dentate gyrus. *Neurosci Lett* **43:** 49–54, 1983.
14. Douglas, R. M. Long lasting synaptic potentiation in the rat dentate gyrus following brief high frequency stimulation. *Brain Res* **126:** 361–365, 1977.
15. Douglas, R. M. and G. V. Goodard. Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. *Brain Res* **86:** 205–215, 1975.
16. Dragunon, M. and G. V. Goddard. Adenosine modulation of amygdala kindling. *Exp Neurol* **84:** 654–665, 1984.
17. Dunwiddie, T. V. Endogenously released adenosine regulates excitability in the *in vitro* hippocampus. *Epilepsia* **21:** 541–548, 1980.
18. Dyer, R. S. and W. K. Boyes. Trimethyltin reduces recurrent inhibition in rats. *Neurobehav Toxicol Teratol* **6:** 369–371, 1984.
19. Giacchino, J. L., G. G. Somjen, D. P. Frush and J. O. McNamara. Lateral entorhinal cortical kindling can be established without potentiation of the entorhinal-granule cell synapse. *Exp Neurol* **86:** 483–492, 1984.
20. Hjorth-Simonsen, A. Projection of the lateral part of the entorhinal area to the hippocampus and fascia dentata. *J Comp Neurol* **146:** 219–232, 1972.
21. Joy, R. M. and T. E. Albertson. Effects of lindame on excitation and inhibition evoked in dentate gyrus by perforant path stimulation. *Neurobehav Toxicol Teratol* **7:** 1–8, 1985.
22. Joy, R. M. and T. E. Albertson. Lindane does not antagonize recurrent collateral inhibition in dentate gyrus. *Toxicologist* **5:** 85, 1985.
23. Kairiss, E. W., R. J. Racine and G. K. Smith. The development of the interictal spike during kindling in the rat. *Brain Res* **322:** 101–110, 1984.
24. Krug, M., A. N. Chepkova, C. Geyer and T. Ott. Aminergic blockade modulates long-term potentiation in the dentate gyrus of freely moving rats. *Brain Res Bull* **11:** 1–6, 1983.
25. Lee, K. S., M. Reddington, P. Schubert and G. Kreutzberg. Regulation of the strength of adenosine modulation in the hippocampus by a differential distribution of the density of A_1 receptors. *Brain Res* **260:** 156–159, 1983.
26. Lee, K. S., P. Schubert, H. Emmert and G. W. Kreutzberg. Effect of adenosine versus adenine nucleotides on evoked potentials in a rat hippocampal slice preparation. *Neurosci Lett* **23:** 309–314, 1981.
27. Lee, K., P. Schubert, V. Gribkoff, B. Sherman and G. Lynch. A combined *in vivo/in vitro* study of the presynaptic release of adenosine derivatives in the hippocampus. *J Neurochem* **38:** 80–83, 1982.
28. Lee, K. S., P. Schubert and H. Heinemann. The anticonvulsive action of adenosine: A postsynaptic, dendritic action by a possible endogenous anticonvulsant. *Brain Res* **321:** 160–164, 1984.
29. Lee, K. S., P. S. Schubert, M. Reddington and G. W. Kreutzberg. Adenosine receptor density and the depression of evoked neuronal activity in the rat hippocampus *in vitro*. *Neurosci Lett* **37:** 81–85, 1983.
30. Lomo, T. Patterns of activation in a monosynaptic cortical pathway: The perforant path input to the dentate area of the hippocampal formation. *Exp Brain Res* **12:** 18–45, 1971.
31. Lynch, G. and M. Baudry. The biochemistry of memory: A new and specific hypothesis. *Science* **224:** 1057–1063, 1984.

32. 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.
33. 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.
34. Maru, E., J. Tatsuno, J. Okamato and H. Ashida. Development and reduction of synaptic potentiation induced by perforant path kindling. *Exp Neurol* **78:** 409–424, 1982.
35. McNaughton, B. I. and C. A. Barnes. Physiological identification and analysis of dentate granule cell responses to stimulation of the medial and lateral perforant pathways in the rat. *J Comp Neurol* **175:** 439–454, 1977.
36. Phillis, J. W. and P. H. Wu. The role of adenosine and its nucleotides in central synaptic transmission. *Prog Neurobiol* **16:** 187–239, 1981.
37. Proctor, W. R. and T. V. Dunwiddie. Adenosine inhibits calcium spikes in hippocampal pyramidal neurons *in vitro*. *Neurosci Lett* **35:** 197–201, 1983.
38. Racine, R. J. and N. W. Milgram. Short-term potentiation phenomena in the rat limbic system. *Brain Res* **260:** 201–216, 1983.
39. Racine, R. J., J. G. Gartner and W. M. Burnham. Epileptiform activity and neural plasticity in limbic system structures. *Brain Res* **47:** 262–268, 1972.
40. Racine, R. J., N. W. Milgram and S. Hafner. Long-term potentiation phenomena in the rat limbic forebrain. *Brain Res* **260:** 217–231, 1983.
41. Reddington, M., K. S. Lee and P. Schubert. An A_1-adenosine receptor, characterized by [H^3] cyclohexyladenosine binding, mediates the depression of evoked potentials in a rat hippocampal slice preparation. *Neurosci Lett* **28:** 275–279, 1982.
42. Schubert, P. and U. Mitzdorf. Analysis and quantitative evaluation of the depressive effect of adenosine on evoked potentials in hippocampal slices. *Brain Res* **172:** 186–190, 1979.
43. Segal, M. Intracellular analysis of a postsynaptic action of adenosine in the rat hippocampus. *Eur J Pharmacol* **79:** 193–199, 1982.
44. Sloviter, R. S. and B. P. Damiano. On the relationship between kainic acid-induced epileptiform activity and hippocampal neuronal damage. *Neuropharmacology* **20:** 1003–1011, 1981.
45. Storm-Mathisen, J. Localization of transmitter candidates in the brain: The hippocampal formation as a model. *Prog Neurobiol* **8:** 119–181, 1977.
46. Tuff, L. P., R. J. Racine and R. Adamec. The effects of kindling on GABA-mediated inhibition in the dentate gyrus of the rat. I. Paired-pulse depression. *Brain Res* **277:** 79–90, 1983.
47. Walker, J. E. Effect of aminophylline on seizure thresholds and brain regional cyclic nucleotides in the rat. *Exp Neurol* **74:** 299–304, 1981.
48. Winson, J. Influence of raphe nuclei on neuronal transmission from perforant pathway through dentate gyrus. *J Neurophysiol* **44:** 937–950, 1980.