Pharmacology Biochemistry & Behavior, Vol. 19, pp. 339–343, 1983. © Ankho International Inc. Printed in the U.S.A.

Caffeine Modification of Kindled Amygdaloid Seizures

T. E. ALBERTSON,*§ R. M. JOY†‡§ AND L. G. STARK†§

Departments of Internal Medicine, Pharmacology,† Veterinary Pharmacology and Toxicology‡ and Health Sciences Neurotoxocology Unit,§ University of California, Davis, CA 95616*

Received 6 December 1982

ALBERTSON, T. E., R. M. JOY AND L. G. STARK. *Caffeine modification of kindled amygdaloid seizures.* PHARMACOL BIOCHEM BEHAV **19**(2) 339–343, 1983.—Rats were kindled during exposure to caffeine (50 mg/kg) or saline given IP twenty minutes before daily electrical stimulation of the amygdala until 3 kindled amygdaloid seizures (KAS) occurred. They were then stimulated for 3 days without drug pretreatment followed by 5 additional days with drug pretreatment. There were no significant differences between the two groups in the number of daily stimulations or in the total seconds of cumulative afterdischarge (AD) needed to reach the first KAS. During kindling, the daily average AD tended to be longer in the caffeine treated group. This difference became significant (>200% saline) when the KAS was reached. When KAS animals were stimulated without caffeine pretreatment, the average AD returned to control lengths. When put back on caffeine pretreatment, the average AD was again increased. Caffeine (6–50 mg/kg, IP) was also evaluated in previously kindled rats using suprathreshold (400 μAMP) and threshold (20 μA increments) seizures. Caffeine had no consistent effect on threshold values. However, 12–50 mg/kg of caffeine increased seizure severity and AD durations after threshold stimulation. With suprathreshold stimulation, the length of the AD was significantly increased only after the highest dose of caffeine. It would appear that caffeine lengthens induced afterdischarges both during the acquisition phase of kindling and in the fully kindled subject. Caffeine does not appear to lower seizure thresholds or increase the rate of acquisition of the KAS in the doses tested in this model. It is postulated that caffeine may modify the KAS through an inhibition of the mechanisms which terminate the elicited AD.

Seizure threshold Caffeine Limbic system Kindled amygdaloid Seizure Rat

THE METHYLXANTHINE alkaloid caffeine is widely used in our society. The average daily intake of caffeine in some age groups is as high as 0.63 mg/kg/day with 99th percentile consumption levels of 3.3 mg/kg/day [14]. Despite the widespread usage of caffeine, very little work has been done exploring its interactions with epilepsy.

The neurotoxic effects of caffeine are obvious at high doses. Rats given 100–200 mg/kg IP of caffeine show hyperexcitability, increased spontaneous activity, piloerection and intermittent body jerks. With doses of caffeine greater than 150 mg/kg, spontaneous generalized seizures occur. Doses of caffeine above 300 mg/kg produce seizure status leading to death [7]. Few studies report on the effects of low level caffeine exposures. Caffeine has been reported to increase cortical AD durations in intact curarized and *cerveau isolé* cats after 5 mg/kg IV [8].

The kindling model of epilepsy [9] has been used in this laboratory to examine the proconvulsant effects of several agents including pentylenetetrazol, bicuculline, strychnine, dieldrin and lindane [1, 2, 4, 5, 10, 11]. The acquisition phase of the kindling model of epilepsy is particularly sensitive to proconvulsant effects [4, 5, 10, 11]. Suprathreshold and threshold stimulations in fully kindled animals have also been used to explore for proconvulsant effects of compounds [2]. In this study, we report the effects of caffeine on the acquisition of the kindled amygdaloid response and on seizure expression in fully kindled subjects.

METHOD

Subjects

Male Sprague-Dawley rats, weighing from 300–325 g, were the subjects. They were housed individually with free access to food and water. A constant 7 a.m. to 7 p.m. light-dark cycle was maintained.

Preparation of Subjects for Amygdaloid Stimulation

All 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 ga 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 intra-aural line). Stainless steel screws were placed over the 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 amygdala, 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 10 days

to recover from the surgical procedures before being used in any of the experiments.

Procedure Used to Produce Amygdaloid Kindling

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 S-44 stimulator and delivered to the amygdala through a constant current output. The stimulus consisted of a one second train of 60 Hz biphasic square waves, each 1 msec in duration and 400 μAMP in amplitude. At the termination of the stimulus train, the amygdala and the EEG electrodes were electronically switched to a Grass Model 7 polygraph. Electrical activity from the amygdala and the cortex was recorded until all evidence of seizure activity had ceased.

Two measurements of seizure severity were employed. The first was the duration of afterdischarge (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 1 per second or faster, in amygdala and/or cortex. If additional AD occurred within one minute of the termination of the preceeding 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 [16] was used in which a score of (0) was assigned for no behavioral response; (1) indicated facial clonus; (2) indicated 1 plus head nodding or head and neck clonus; (3) indicated 2 plus forelimb clonus; (4) indicated 3 plus rearing; and (5) indicated rearing and falling over onto the cage floor.

Experiment 1—Caffeine and the Acquisition of Kindled Seizures

Ten days after surgery, rats were given daily intraperitoneal injections of saline (1 ml/kg) (N=11) or caffeine (N=11) (50 mg/kg as a 1 ml/kg solution). Electrical stimulation of the amygdala (400 μA) occurred twenty minutes later. Each rat was injected and stimulated daily until 3 kindled amygdaloid seizures (KAS) were produced (stage 5 rank). Then the rats were stimulated for 3 more days without pretreatment. Finally, rats were stimulated for 5 more days with saline or caffeine pretreatment.

Experiment 2—Caffeine and Fully Kindled Seizures

Twenty fully kindled rats were used to evaluate the effect of caffeine on KAS expression. The animals had been drug free and shown stable responses to stimulation for over two weeks before testing began. On day 1 rats were given a suprathreshold (400 μAMPs) control stimulation. The next day the animals randomly received saline (ml/kg) or caffeine (6, 12, 25 or 50 mg/kg). They were stimulated (400 μAMPs) twenty minutes later. One or two days later another suprathreshold control stimulation was given. This was followed by another drug day using either suprathreshold or threshold stimulations. This weekly pattern was then repeated. If control data showed any signs of instability, the rat was dropped from the study. Animals were randomly assigned to drug/dose groups and the order of suprathreshold/threshold stimulation was randomly assigned.

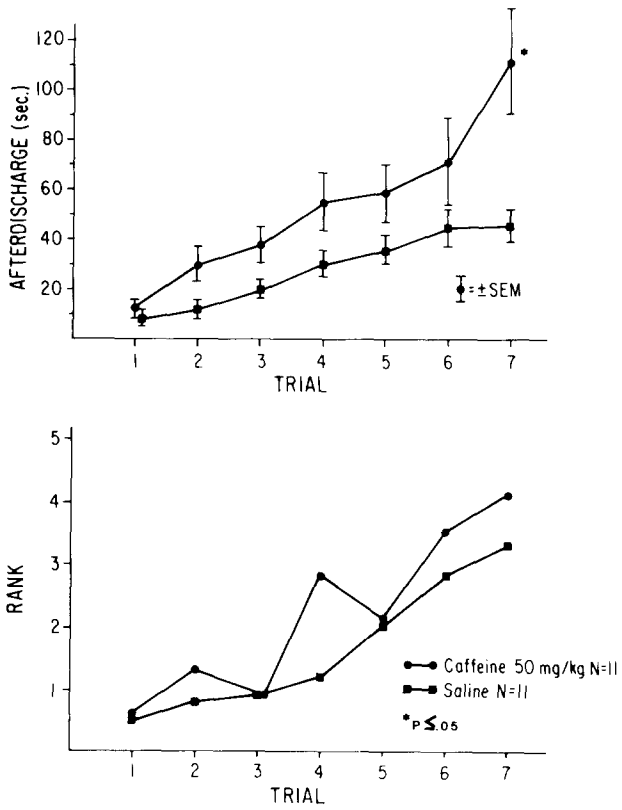

FIG. 1. The effects of daily pretreatment with 50 mg/kg caffeine or saline on the acquisition (afterdischarge duration and behavioral rank) of the kindled amygdaloid seizure response are shown. The number of rats in each group was eleven.

Procedure Used to Determine Seizure Thresholds

Seizure thresholds were determined at 1 minute intervals with increasingly higher current until an AD occurred. The initial current (20 μAmps) was increased by 20 μAmp increments. The threshold was defined to be the lowest current intensity producing an afterdischarge meeting the criteria defined above. In addition to threshold, AD duration and seizure severity were also measured during these stimulations.

Assessment of Effects

All comparisons were made to either saline acquisition group, saline threshold trials or saline suprathreshold group. Parametric measures including number of stimulations to kindle, cumulative seconds of AD to kindle, seizure thresholds and seconds of AD were compared using the Students *t*-test. All non-parametric measures including rank scores and transformations of seizure rank scores were compared using the Mann-Whitney U-test or a signed-ranks test. A repeated measures ANOVA was performed comparing the saline and caffeine acquisition groups.

RESULTS

The effects of daily dosing with 50 mg/kg of caffeine on the acquisition of the kindled amygdaloid seizure in rats is shown in Fig. 1 and Table 1. As can be seen in Fig. 1, the

TABLE 1

THE EFFECT OF CAFFEINE ON THE ACQUISITION OF KINDLED AMYGDALOID SEIZURES

Treatment*	N	Number of Stimulations Until First Stage 5 Seizure	Cumulative Afterdischarge‡ (sec)
Saline (cc/kg)	11	8.5 ± 0.7†	305 ± 53
Caffeine (50 mg/kg)	11	8.2 ± 0.8	471 ± 78

*Treatment was given twenty minutes before daily kindling stimulations.
†Values are means ± S.E.M.
‡The cumulative average afterdischarge duration until first stage 5 seizure.

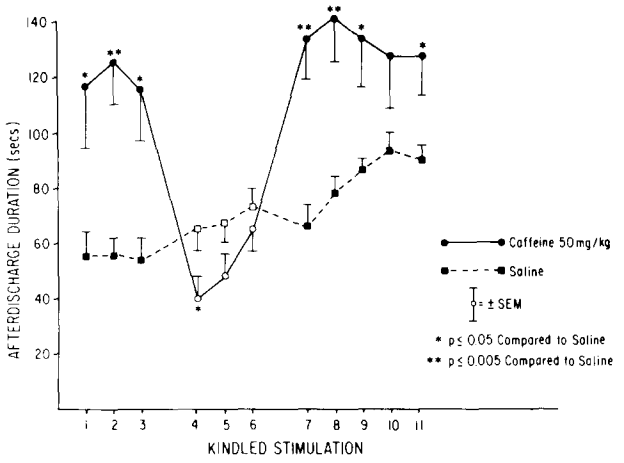

FIG. 2. The effect of daily pretreatment with caffeine or saline on afterdischarge duration during the first 3 elicited kindled amygdaloid seizures (stage 5) is shown (kindled stimulations 1–3). Kindled stimulations 4–6 occurred without caffeine or saline pretreatment (empty circles and squares). Kindled stimulations 7–11 occurred with caffeine or saline pretreatment. All animals exhibited stage 5 seizures for all eleven stimulations.

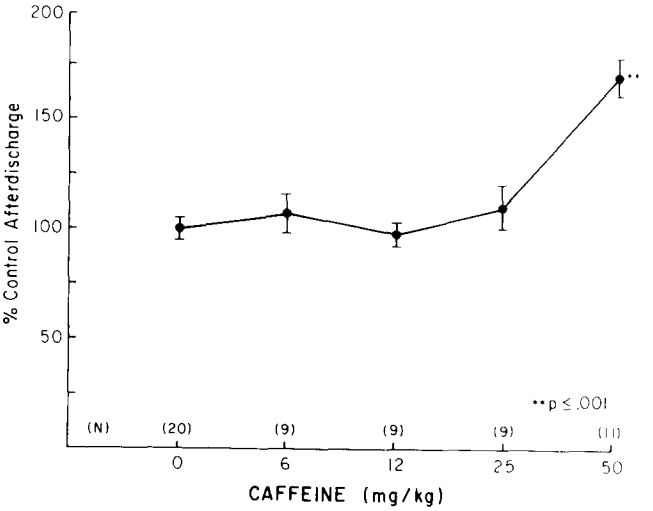

FIG. 3. Caffeine modification of the afterdischarges of kindled amygdaloid seizures after suprathreshold (400 μA) stimulation is shown. (N) equals the number of rats used for each dose tested. The 0 mg/kg caffeine dose received a cc/kg saline. Bars are ±S.E.M.

average daily afterdischarge durations of the caffeine treated animals were consistently longer than the saline treated animals, $F(1,20)=20$, $p\leq 0.01$. This increase reached individual statistical significance by the seventh trial. As expected, a significant ($p\leq 0.01$) trials effect was found; $F(6,120)=14.3$. Also a significant ($p\leq 0.05$) interaction between the effect of caffeine and trials, $F(6,120)=2.5$, was noted. The average number of stimulations to reach the first stage 5 seizure was not significantly different between the caffeine treated and control groups (Table 1). Because the average length of each caffeine AD was longer and the number of stimulations to reach the first KAS was approximately the same, the cumulative seconds of afterdischarge to reach the first KAS were somewhat longer for the caffeine treated group (Table 1).

Figure 2 shows that during the first 3 KAS trials the average AD was approximately 200% that of saline treated animals. When 3 trials were performed without caffeine treatment, the animals previously treated with caffeine still demonstrated fully kindled seizure ranks (stage 5) to the daily stimulation. However, the average AD values decreased to near or below those of the saline group. With resumption of the caffeine pretreatment (kindled trials 7–11), the average AD of the animals increased to the previous lengths.

The effects of various doses of caffeine on stable KAS are shown in Figs. 3 and 4. After suprathreshold stimulation (Fig. 3), caffeine increased AD length only at the 50 mg/kg dose. Seizure ranks remain at stage 5 with all doses tested. At the highest doses tested, the animals showed no prestimulation EEG changes but were very aggressive and hyperresponsive to novel stimulation. With the threshold stimulation paradigm (Fig. 4), caffeine increased AD duration at doses of 12–50 mg/kg. Seizure ranks tended to be more severe while seizure thresholds were unchanged (Fig. 4).

DISCUSSION

The increase in afterdischarge duration found in this study during acquisition and in mature KASs is consistent

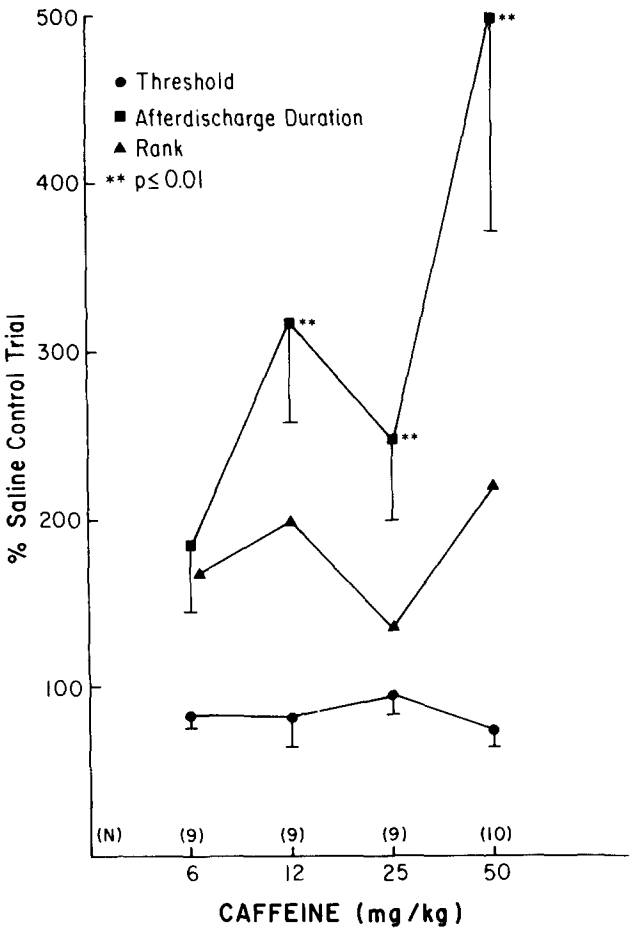

FIG. 4. The modification of kindled amygdaloid threshold seizures by various doses of caffeine is presented. (N) equals the number of rats used for each dose. Bars are ±S.E.M.

with the preliminary finding of Cain with theophylline [6]. The removal of caffeine in this study returned afterdischarge durations down to or lower than control lengths. This effect and the resulting return to longer AD lengths after reinstitution of caffeine indicates that the increase in AD duration is dependent upon the presence of caffeine. This finding stands in contrast to the possibility that caffeine simply accelerated the acquisition of the permanent maximal mature kindled amygdaloid afterdischarge. The decrease in afterdischarge duration to levels significantly less than saline group after discontinuation of daily pretreatments with caffeine may reflect a rebound or withdrawal effect noted previously with caffeine [18].

Daily pretreatment with stimulant, but non-convulsive doses, of caffeine did not increase the rate of acquisition of the kindled amygdaloid seizure in this study. This finding is in contrast to preliminary work reported by Cain in which higher equivalent doses of a methylxanthine were used [6]. In the preliminary work by Cain, rats were stimulated every 48 hours at 30 minutes after pretreatment with either 50 or 100 mg/kg of the methylxanthine theophylline [6]. The slight differences in potency, doses and pharmacokinetics of the two methylxanthines utilized, as well as the difference in stimulation paradigms (daily vs. every 48 hours) may explain the variation in findings between the two studies.

In this study, high doses of caffeine also resulted in prolongation of the afterdischarge duration to either threshold or suprathreshold stimulation in fully kindled rats. When threshold stimulation was used, significant prolongation of the elicited afterdischarge was noted after doses of 12 mg/kg caffeine or greater. Although a tendency for more severe seizures was noted, no significant change in seizure threshold was seen. Thus, caffeine appears to be proconvulsant by prolonging and potentiating elicited electrical activity and not by lowering threshold or accelerating acquisition.

One explanation for these findings would be that caffeine and the other methylxanthines interfere with the processes which terminate electrical seizure activity. Possible mechanisms by which methylxanthines could modulate kindled amygdaloid seizures include their blockade of adenosine receptors, inhibition of phosphodiesterase activity, translocation of intracellular calcium and inhibition of benzodiazepine receptor binding [12, 13, 15, 17]. Concentrations high enough to affect translocation of intracellular calcium are probably not reached in the central nervous system [15]. Doses of 50–100 mg/kg of caffeine or theophylline are required to inhibit brain phosphodiesterase and to alter brain cyclic nucleotide concentrations by this mechanism [15]. Although a 10 mg/kg dose of theophylline lowered cyclic GMP brain levels while it had no effect on cyclic AMP levels at 30 min, 100 mg/kg of theophylline was needed to significantly raise both cyclic GMP and cyclic AMP brain levels at 30 min [17]. The decrease in the level of brain cyclic GMP after the lower dose of theophylline is thought to be from its effect on the adenosine receptor [17]. Thus, adenosine receptor blockade is thought to be the most sensitive mechanism by which the methylxanthines act [15].

In preliminary work, rats given either daily intraventricular infusions of analogs or derivatives of either cyclic AMP or cyclic GMP (2′-o-dibutyrl-cAMP and 8-bromo-cGMP) have been shown to develop kindled amygdaloid seizures more rapidly than control infused rats [6]. The mechanism by which these seemingly competitive nucleotides could both result in acceleration of the acquisition of the kindled seizure is unclear. Caffeine and theophylline have also been shown to competitively inhibit benzodiazepine binding [13] whose agonists protect against caffeine induced seizures [12]. However, recent *in vitro* studies have shown that caffeine and theophylline are more potent inhibitors of adenosine receptor binding than of endogenous benzodiazepine receptor binding. This suggests that the adenosine receptor is a better candidate than the benzodiazepine receptor for methylxanthine mediation [12]. Supporting this view is the fact that the benzodiazepine receptor antagonist Ro-15-1788 has been shown not to potentiate kindled amygdaloid seizures [1].

This study has shown the ability of the methylxanthine caffeine to prolong the afterdischarge duration of amygdaloid kindled seizures after threshold and suprathreshold stimulation. In addition, the afterdischarge duration has been prolonged during the acquisition of the kindled seizure only when caffeine pretreatment is present. Although the doses which prolonged afterdischarge duration in this animal model of epilepsy are far greater than those generally consumed by humans, the possibility that caffeine may potentiate human seizures needs further investigation.

ACKNOWLEDGEMENTS

We thank P. Wong and K. Ojakangas for their technical assistance and D. Case for preparation of this manuscript. This work was supported in part by BRS Grant No. 2507RR05457 and by a Northern California Occupational Health Center grant to T.E.A.

REFERENCES

1. Albertson, T. E., J. F. Bowyer and M. G. Paule. Modification of the anticonvulsant efficacy of diazepam by Ro-15-1788 in the kindled amygdaloid seizure model. *Life Sci* **31:** 1597–1601, 1982.
2. Albertson, T. E., L. G. Stark and R. M. Joy. The effects of doxapram, diazepam, phenobarbital and pentylenetetrazol on suprathreshold and threshold stimulations in amygdaloid kindled rats. *Neuropharmacology* **22:** 245–248, 1983.
3. Boulenger, J.-P., J. Patel and P. J. Marangos. Effects of caffeine and theophylline on adenosine and benzodiazepine receptors in human brain. *Neurosci Lett* **30:** 161–166, 1982.
4. 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.
5. Bowyer, J. F., W. D. Winters and T. E. Ablertson. Pentylenetetrazol augmentation of developing kindled amygdaloid seizures. *Neuropharmacology* **20:** 1225–1227, 1981.
6. 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.
7. Chu, N.-S. Caffeine- and aminophylline-induced seizures. *Epilepsia* **22:** 85–94, 1981.
8. Funderburk, W. H. and R. T. Woodcock. Comparative effects of analeptic drugs on the central nervous system. *J Pharmacol Exp Ther* **119:** 148, 1957.
9. 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.
10. Joy, R. M., L. G. Stark and T. E. Albertson. Proconvulsant effects of lindane enhancement of amygdaloid kindling in the rat. *Neurobehav Toxicol Teratol* **4:** 347–354, 1982.
11. Joy, R. M., L. G. Stark, S. L. Peterson, J. F. Bowyer and T. E. Albertson. The kindled seizure production of and modification by dieldrin in rats. *Neurobehav Toxicol Teratol* **2:** 117–124, 1980.
12. Marangos, P. J., A. M. Martino, S. M. Paul and P. S. Kolnick. The benzodizepines and inosine antagonize caffeine-induced seizures. *Psychopharmacology (Berlin)* **72:** 269–273, 1981.
13. 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.
14. Oser, B. L. and R. A. Ford. Caffeine: An update. *Drug Chem Toxicol* **4:** 311–329, 1981.
15. Phillis, J. W. and P. H. Wu. The role of adenosine and its nucleotides in central synaptic transmission. *Prog Neurobiol* **16:** 187–239, 1981.
16. Racine, R. J. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* **32:** 281–294, 1972.
17. Stefanovich, V. Influence of theophylline on concentrations of cyclic 3′,5′-adenosine monophosphate and cyclic 3′,5′-guanosine monophosphate of rat brain. *Neurochem Res* **4:** 587–594, 1979.
18. Vitiello, M. V. and S. C. Woods. Evidence for withdrawal from caffeine by rats. *Pharmacol Biochem Behav* **6:** 553–555, 1977.