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Rapid Development of Functional Tolerance to Caffeine-Induced Seizures in Rats (42726)

MASATO YASUHARA AND GERHARD LEVY

Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, New York 14260

Abstract. The concentration and time dependence of caffeine-induced neurotoxicity was determined by infusing rats intravenously with caffeine at a rate of about 5, 12.5, and 25 mg kg^{-1} min^{-1} until the onset of generalized seizures which occurred at about 82, 28, and 11 min, respectively. The concentration of caffeine in the serum, brain, and cerebrospinal fluid at onset of seizures increased with decreasing infusion rate; the concentrations of caffeine metabolites were negligible and serum protein binding was not affected by the infusion rate. In another experiment, one group of rats was infused with caffeine for 60 min at about 2.2 mg kg^{-1} min^{-1} whereas another group was infused with solvent only. Both groups were then immediately infused with caffeine at about 22 mg kg^{-1} min^{-1} until onset of seizures. Caffeine concentrations at that time in serum, brain, and cerebrospinal fluid were significantly higher in the caffeine-pretreated animals than in the solvent-pretreated controls. The same pretreatment 17 hr before the fast infusion of caffeine had no apparent effect on caffeine concentrations at onset of seizures. These results show that functional tolerance to the seizure-inducing effect of caffeine in rats develops within minutes and that it is reversible within hours or less.

The central nervous system stimulant drug caffeine can cause convulsions when administered in very large doses to animals (1) or humans (2). In smaller doses, it has been used to increase the duration of seizures in patients on electroconvulsive therapy (3). Theophylline, another methylxanthine drug, is widely used for the treatment of asthma and neonatal apnea (4). Overdoses of theophylline cause neurotoxicity including life-threatening seizures which have occurred in many patients (5, 6). Administration of caffeine can reduce the concentrations of theophylline required to produce seizures (7). We describe here a pharmacodynamic study of experimental caffeine neurotoxicity (as reflected by the occurrence of seizures), with emphasis on the role of rate of drug administration and drug concentration, which has yielded evidence of rapid development of functional tolerance. These findings are considered important because previous investigations of tolerance development to the pharmacologic effects of caffeine have focused on the stimulant rather than the neurotoxic effect of the drug. There is evidence, to be reviewed in the Discussion, that these effects may be mediated by different kinds of receptors.

Methods. Inbred, male Lewis rats (LEW/CrlBR, Charles River Breeding Laboratories, Wilmington, MA), maintained on Charles River Rat–Mouse–Hamster Formula, were used in this investigation. The animals were allowed 1 to 2 weeks to adjust to the local animal facilities and to recover from possible stress incurred during transport. Before the investigation, the rats were housed individually in metal cages in an environment of controlled temperature (23–25°C) and alternating 12 hr light (7 AM–7 PM) and dark cycles. Food and water were withdrawn in the morning on the day of the experiment. A Silastic cannula was implanted in the right external jugular vein of each animal under light ether anesthesia 1 or 2 days before the pharmacodynamic study.

The effect of infusion rate on the concentrations of caffeine at onset of maximal seizures was determined in groups of 10 rats who received an iv infusion of caffeine (as caffeine citrate; Pfaltz & Bauer, Waterbury, CT) at one of three rates (1.03, 2.55, or 5.15 mg/min) by means of a Harvard infusion pump. The infusion solution consisted of caffeine citrate equivalent to 50 mg of caffeine in 1 ml of distilled water. Onset of maximal seizures was evidenced by tonic ex-

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tension of all four legs to the rear. The caffeine infusion was stopped immediately at onset of maximal seizures and samples of CSF, blood, and brain were obtained, in that order. CSF was obtained from the cisterna magna, as much blood as possible was withdrawn from the abdominal aorta, and the animals were then decapitated by guillotine and the brain was removed. The biologic samples were stored in a freezer pending assay.

The effect of caffeine pretreatment on the concentrations of caffeine at onset of maximal seizures was determined in two separate experiments on rats obtained in different shipments. In the first experiment, one group of rats was infused with caffeine for 60 min at a rate of 0.515 mg/min and another group was infused with solvent only (citric acid solution). Both groups were then immediately infused with caffeine at a rate of 5.15 mg/min until onset of seizures. Samples of CSF, blood, and brain were obtained at that time. In the second experiment, the same pretreatment was given 17 hr before the rapid infusion of caffeine (5.15 mg/min). Blood samples (0.2 ml) were obtained at the end of pretreatment and just before starting the rapid caffeine infusion.

All the experiments were scheduled such that maximal seizures occurred between 9 AM and 12 noon. Body temperature was maintained in all animals by placing the rats on isothermal pads (Deltaphase; Braintree Scientific Inc., Braintree, MA).

The protein binding of caffeine was determined by equilibrium dialysis of serum (0.3 ml) against an equal volume of 0.13 *M* sodium and potassium phosphate buffer, pH 7.4, containing caffeine, 300 mg/liter. The dialysis was performed at 37°C for 3 hr in Plexiglas cells separated by a cellulose membrane with a molecular exclusion limit of 12,000 to 14,000 Da (Visking cellulose tubing; Union Carbide Corp., New York, NY).

The concentrations of caffeine and its metabolites (theophylline, theobromine, 1-methyluric acid, and 1,3-dimethyluric acid) in serum, dialysis buffer solution, CSF, and brain were determined by the high-performance liquid chromatographic method of Ramzan and Levy (5) except for changing the internal standard to β-hydroxyethyltheophylline (Sigma Chemical Co., St. Louis, MO). The detection limit for these compounds was between 0.5 and 2 mg/liter. The presence of paraxanthine in serum and CSF samples was checked by an ion-pair high-performance liquid chromatographic method (8). The detection limit for paraxanthine was 2 mg/liter.

The administered doses of caffeine were calculated as the product of the infusion time and the rate of infusion and were normalized for body weight. The results of the experiment on the effect of different infusion rates were analyzed by one-way analysis of variance followed by the Newman–Keuls test where appropriate. Bartlett's test was used to assess the homogeneity of variances. The Kruskal–Wallis test followed by a nonparametric multiple comparison analysis (9) was used in case of heteroscedasticity. The statistical significance of differences between two groups was determined by Student's *t* test or by the nonparametric Mann–Whitney *U* test when the variances were unequal.

Results. The results of the experiment designed to determine the effect of caffeine infusion rate on the drug concentrations at onset of seizures in rats are summarized in Table I. Depending on the infusion rate, seizures occurred in about 11 to 82 min. The total infused dose of caffeine and the concentrations of the drug in the serum, brain, and CSF increased with decreasing infusion rate. The protein binding of caffeine in serum was only about 10% and was not affected by the infusion rate. Metabolites of caffeine were either nondetectable or present in very low concentrations. Summarized here are the relevant findings for the slowest infusion rate which yielded the highest metabolite concentrations: theophylline, 3.0 ± 0.4 mg/liter in serum (mean ± SD) and lower concentrations in brain and CSF; theobromine, 1.1 ± 0.2 mg/liter in serum of 5 rats and detectable in serum of 4 other rats and in CSF of 6 out of 10 rats; 1,7-dimethylxanthine, detectable in serum of 3 out of 10 rats but not in CSF; 1-methyluric acid and 1,3-dimethyluric acid, not detectable.

Pretreatment with caffeine for 60 min by infusion at a very slow rate increased the serum, brain, and CSF concentrations of the drug at onset of seizures produced by an im-

TABLE I. EFFECT OF INFUSION RATE ON CONCENTRATIONS OF CAFFEINE AT ONSET OF MAXIMAL SEIZURES IN RATS[a]

Parameter	Infusion rate (mg/min) 1.03	2.55	5.15
Body weight (g)	199 ± 11	198 ± 12	196 ± 13
Infusion time (min)[b]	82 ± 6*	28 ± 3*	11 ± 1*
Total dose (mg/kg)[b]	426 ± 30*	361 ± 23*	281 ± 20*
Serum concn (mg/liter)			
Total drug[b]	456 ± 20	424 ± 18	400 ± 39†
Free drug[b]	415 ± 22*	384 ± 18*	358 ± 39[d,*]
Brain concn (mg/kg)[c]	408 ± 23	396 ± 16[d]	381 ± 18†
CSF concn (mg/liter)[b]	428 ± 20*	399 ± 18*	348 ± 18[d,*]
Free fraction in serum × 100	91.0 ± 1.5	90.7 ± 1.3	89.8 ± 1.2[d]

[a] Results are reported as mean ± SD, $n = 10$.
[b] Infusion rate had a significant effect on the results ($P < 0.001$ by one-way analysis of variance).
[c] Infusion rate had a significant effect on the results ($P < 0.025$ by one-way analysis of variance).
[d] $n = 9$.
* Significantly different from the results of the other two groups ($P < 0.05$).
† Significantly different from the results of the slowest infusion rate group ($P < 0.025$).

mediately following rapid infusion of the drug (Table II). When the rapid infusion was administered 17 hr after pretreatment rather than immediately thereafter, there was no significant difference in caffeine concentrations between pretreated and control animals (Table III). The shorter infusion time for the caffeine-pretreated rats was due to the presence of appreciable caffeine from the slow infusion administered 17 hr earlier (31 ± 11 mg/liter in serum).

The concentrations of caffeine metabolites in the pretreated rats with immediately following rapid infusion were similar to the concentrations summarized in the preceding paragraph. Metabolite concentrations (mg/liter in serum and CSF) were somewhat higher in rats pretreated 17 hr earlier: theophylline, 12.5 ± 1.5 and 8.2 ± 0.8; theobromine, 7.8 ± 1.0 and 6.5 ± 0.8. No metabolites were detected in the control animals.

Discussion. The pharmacodynamic implications of the infusion rate experiment are best discussed by considering the results of an earlier, similar study of the convulsive effect of theophylline in rats (5). In that study, drug concentrations in serum and brain at onset of seizures increased with *increasing*

TABLE II. EFFECT OF CAFFEINE PRETREATMENT ON CONCENTRATIONS OF CAFFEINE AT ONSET OF MAXIMAL SEIZURES IN RATS RECEIVING A SUBSEQUENT RAPID INFUSION OF THE DRUG[a]

Parameter	Control	Caffeine pretreated
Body weight (g)	226 ± 5	230 ± 9
Infusion time (min)	13.3 ± 0.8	8.3 ± 0.8**
Total dose (mg/kg)	303 ± 15	320 ± 15[b]
Serum concn (mg/liter)		
Total drug	428 ± 12	460 ± 25*
Free drug	378 ± 13	405 ± 28
Brain concn (mg/kg)	423 ± 10	452 ± 10**
CSF concn (mg/liter)	392 ± 14	411 ± 11*
Free fraction in serum × 100	88.4 ± 0.9	88.0 ± 1.7

[a] Rats were infused iv with citric acid (control) or caffeine at a rate of 0.515 mg/min for 60 min just before starting the rapid caffeine infusion (5.15 mg/min). Results are reported as mean ± SD, $n = 6$.
[b] The sum of the total amounts administered by pretreatment and subsequent rapid infusion of caffeine.
* Significantly different from control group, $P < 0.05$; ** $P < 0.001$.

TABLE III. EFFECT OF CAFFEINE PRETREATMENT ON CONCENTRATIONS OF CAFFEINE AT ONSET OF MAXIMAL SEIZURES IN RATS RECEIVING A RAPID INFUSION OF THE DRUG ON THE NEXT DAY[a]

Parameter	Control	Caffeine pretreated
Body weight (g)	202 ± 8	192 ± 9*
Infusion time (min)	11.6 ± 0.9	10.4 ± 1.0*
Dose (mg/kg)[b]	295 ± 15	279 ± 22
Serum concn (mg/liter)		
Total drug	393 ± 11	408 ± 35[c]
Free drug	351 ± 11	360 ± 33
Brain concn (mg/kg)	406 ± 8	407 ± 13
CSF concn (mg/liter)	361 ± 6	356 ± 20
Free fraction in serum × 100	89.3 ± 0.6	88.1 ± 1.0

[a] Rats were infused iv with citric acid solution (control) or caffeine at a rate of 0.515 mg/min for 60 min. Seventeen hours later, caffeine was infused at a rate of 5.15 mg/min until onset of maximal seizures. Results are reported as mean ± SD, $n = 10$.

[b] The amount administered at an infusion rate of 5.15 mg/min.

[c] Serum concentrations of caffeine at the end of pretreatment and 17 hr later were 165 ± 19 and 31 ± 11 mg/liter, respectively.

* Significantly different from control group, $P < 0.05$.

infusion rate whereas theophylline concentrations in the CSF were independent of infusion rate. That is the classic situation when the drug distributes relatively slowly from plasma to the site of action, drug distribution between the CSF and the site of action is very rapid, drug metabolites are inactive or present in subeffective concentrations, and development of functional tolerance is absent or negligible under the experimental conditions (10). In the case of caffeine, drug concentrations at onset of seizures increased with *decreasing* infusion rate. Slow drug distribution to the site of action or accumulation of active (agonistic) metabolites would have the opposite effect. On the other hand, an increase in the concentration of a drug required to elicit a defined pharmacologic effect with increasing time of exposure to the drug is characteristic of functional tolerance.

Development of functional tolerance to the seizure-inducing effect of caffeine was also evident in the experiment in which the rats were pretreated with a slow infusion of caffeine immediately before infusion at a faster rate that caused seizures. The caffeine concentrations at onset of seizures were slightly but statistically significantly higher in these animals than in control rats that received only the fast infusion. Thus, a modest degree of functional tolerance developed during 60 min of exposure to even relatively low concentrations of the drug. On the other hand, when the pretreatment occurred 17 hr earlier, there were no significant differences between pretreated and control rats with respect to caffeine concentrations at onset of seizures. This suggests that functional tolerance to the seizure-inducing effects of caffeine is reversible within hours. This conclusion could be challenged because theophylline concentrations, which were quite low but not negligible in this experiment, were not taken into consideration. Based on CSF concentrations at onset of seizures, theophylline is about twice as potent as caffeine (7). Addition of the potency-normalized theophylline concentrations to the caffeine concentrations yields a total CSF concentration of 373 ± 21 mg/liter caffeine equivalent in the rats pretreated 17 hr earlier whereas the corresponding concentration in control animals remained at 361 ± 6 mg/liter since metabolite concentrations were not detectable in the control rats. There is no statistically significant difference between these concentrations.

There have been previous studies of tolerance development to the pharmacologic actions of caffeine but they have not been concerned with the drug's neurotoxicity. In healthy humans, the blood pressure increase associated with acute caffeine administration diminishes and then disappears within 3 days of continued caffeine intake of 250 mg three times a day (11). In animals, tolerance de-

velops within 1 or more days to the psychomotor stimulant effect of caffeine in squirrel monkeys (12), the hypothermic effect in rats (13), and the stimulation of locomotor activity in rats (14). These experiments were not designed to determine if tolerance develops earlier than within 1 or more days and the majority of studies did not permit distinction between metabolic and functional tolerance because drug concentrations were not determined. However, it is generally believed that, since caffeine is an antagonist of adenosine at the receptor level, the central stimulant effects of the drug are due to competitive antagonism of adenosine receptors in the brain and that development of functional tolerance to these effects is due to up-regulation of these receptors (15, 16). Such up-regulation, without any concomitant change in receptor affinity, has been produced by pretreatment of rats with theophylline (16) and of mice with caffeine (17).

There now is strong evidence that the *convulsant* effect of caffeine may be mediated through central benzodiazepine receptors (18, 19). Benzodiazepines inhibit caffeine-induced seizures with a rank order potency that parallels their *in vitro* affinities for the CNS benzodiazepine receptor (20). Caffeine competitively inhibits diazepam and flunitrazepam binding to the benzodiazepine receptor complex (20, 21). Treatment of rats with caffeine produces up-regulation of benzodiazepine binding sites in the brain (22). Even if adenosine receptors play a role in caffeine-induced seizures, these receptors are also subject to up-regulation by exposure to caffeine. Thus, development of functional tolerance to the seizure-inducing effect of caffeine, as observed in our investigation, is consistent in principle with the available knowledge of relevant receptor pharmacology.

Much higher caffeine concentrations are required to alter binding to benzodiazepine receptors than to adenosine receptors (23). Tolerance to the spontaneous locomotor activity stimulating effect of caffeine and up-regulation of adenosine A_1 receptors in rats have been produced by caffeine doses as low as 5 mg kg^{-1} day^{-1} for 2 weeks (15). Up-regulation of benzodiazepine receptors in rats by caffeine apparently required much higher doses, i.e., 75 mg kg^{-1} day^{-1} for 12 days (22). Significantly, determinations of tolerance development and receptor binding changes were made 18 or 27 hr after the last dose of caffeine (15, 22), i.e., a time interval equal to or longer than the 17-hr period during which tolerance was reversed in the present investigation. The rapid onset and offset of tolerance evident in our study are compatible with a possible role of an antagonistic metabolite of caffeine. In fact, paraxanthine (1,7-dimethylxanthine), one of the major metabolites of caffeine (24), exhibits a biphasic effect on locomotor activity in mice, causing depression at lower doses and stimulation at higher doses (23). The role of this metabolite in the rapid development and disappearance of functional tolerance to the seizure-inducing effect of caffeine is being investigated.

Considering the available evidence that the CNS stimulation and improvement of impaired pulmonary function by the methylxanthines are mediated by adenosine, or other, receptors but not by benzodiazepine receptors, whereas benzodiazepine receptors are involved in the convulsant effect of these drugs (21), it becomes apparent that functional tolerance to the different pharmacologic actions of caffeine can develop at different rates. It is of particular interest to determine why the functional tolerance to the seizure-inducing effect of caffeine in rats develops within minutes whereas no such phenomenon has been observed with respect to theophylline (5). From a clinical perspective, it is of interest to determine if preexposure to caffeine can lead to development of functional tolerance to the neurotoxic effects of the widely used bronchodilator drug theophylline.

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