Effect of Chronic Caffeine Administration on Theophylline Concentrations Required to Produce Seizures in Rats (43027)

JIANGUO ZHI AND GERHARD LEVY[1]
Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, New York 14260

Abstract. **Caffeine as well as the antiasthmatic drug theophylline can cause seizures when administered to humans or animals in excessive doses. Studies on rats have shown rapid development of functional tolerance to caffeine-induced seizures whereas repeated pretreatment with theophylline had no significant effect on the theophylline concentrations required to produce seizures. The purpose of this investigation was to determine whether chronic exposure to caffeine can affect susceptibility to the convulsant effect of theophylline. Rats received caffeine, 40 mg/kg, or solvent twice a day for 7 days as an intravenous injection. On the eighth day, theophylline was infused intravenously until the onset of maximal seizures. At this pharmacologic end point, rats pretreated with caffeine had significantly higher theophylline concentrations in the brain and cerebrospinal fluid than did control (solvent-pretreated) animals. Although the concentration differences were relatively small (~11%), they demonstrate in principle the development of caffeine-induced tolerance to the neurotoxic effect of theophylline. Additional experiments showed that the caffeine effect on theophylline neurotoxicity is not acutely mediated by paraxanthine, a major metabolite of caffeine.**

[P.S.E.B.M. 1990, Vol 193]

Caffeine (1,3,7-trimethylxanthine) may be the most widely used drug in western society (1). Its effects on the central nervous system, including (at very high concentrations) the development of seizures, are subject to rapid development of functional tolerance (1–3). Another methylxanthine, theophylline (1,3-dimethylxanthine), is a bronchodilator widely used in the treatment of asthma. A substantial number of individuals on theophylline therapy have experienced neurotoxicity including convulsions. The plasma concentrations of theophylline associated with such convulsions in man vary widely whereas in experimental animals these concentrations vary only slightly under standardized conditions (4). This suggests that patients on theophylline therapy may be subject to certain pharmacodynamically relevant influences that can alter their susceptibility to theophylline-induced neurotoxicity. One such influence may be that of preexposure to caffeine contained in certain beverages and nonprescription drug products.

It has already been established that concomitant administration of caffeine can reduce the concentration of theophylline required to produce convulsions (5). In animal studies, chronic preexposure to pharmacologic (but not toxic) concentrations of theophylline had no significant effect on the concentrations of that drug required to produce seizures, indicating an absence of functional tolerance development (6). However, it is not known whether preexposure to caffeine can produce cross-tolerance to the neurotoxic action of theophylline. Relevant to this issue is the question of why caffeine neurotoxicity is subject to acute functional tolerance development whereas the closely related compound theophylline is not. It has been speculated (2) that paraxanthine (1,7-dimethylxanthine), one of the major metabolites of caffeine (7, 8), may be responsible for this difference in view of its biphasic action on the central nervous system (9).

Materials and Methods

The investigation was conducted on inbred male Lewis rats (Charles River Breeding Laboratories, Wilmington, MA), weighing about 200 g and maintained on Charles River Rat-Mouse-Hamster Formula and water *ad libitum*. After a 1-week period of acclimati-

[1] To whom requests for reprints should be addressed.

Received July 26, 1989. [P.S.E.B.M. 1990, Vol 193]
Accepted November 22, 1989.

0037-9727/90/1933-0210$2.00/0

zation, a Silastic cannula was implanted in the right external jugular vein of each animal under light ether anesthesia and the cannulated rats were transferred to individual metal cages in an environment of constant temperature (25 ± 2°C) and 12:12-hr light:dark cycles. Starting on the next day, two groups of rats received caffeine, 80 mg/kg/day, as a solution of 20 mg/ml as caffeine citrate in sterile normal saline. The drug was injected into the intravenous cannula over 10 sec in two equally divided doses, at 9 AM and 5 PM, for 7 days. Each injection was followed by an equal volume of normal saline. One of the two groups did not receive an injection in the afternoon on the seventh day. A third (control) group of rats received injections of citric acid, 20 mg/ml in sterile normal saline, instead of caffeine citrate. Blood samples (0.3 ml) were obtained at 10 AM on Days 3 and 6 for determination of the serum concentrations of caffeine and its metabolites.

In the morning of the eighth day, the rats were infused with a 40 mg/ml aqueous solution of theophylline, in the form of aminophylline, at a rate of 2.04 mg/min (~10 mg/kg·min) until the onset of maximal seizures (4). During that time the rats were on isothermal pads to maintain normal body temperature. The infusion was stopped at the pharmacologic end point and samples of cerebrospinal fluid (CSF), blood (for serum), and brain were obtained, in that order. The samples were stored at −20°C pending assay within 1 week.

To determine the acute effect of paraxanthine on theophylline-induced seizures, four groups of six jugular vein-cannulated rats received an intravenous injection of paraxanthine in saline solution (2 ml/kg) at a dose of 0, 1, 2, and 4 mg/kg, respectively. Ten minutes later, a 0.3-ml blood sample was obtained. Immediately thereafter, theophylline was infused as described in the preceding paragraph and CSF and blood samples were obtained at the onset of maximal seizures.

CSF, serum, and brain samples were prepared for assay as described previously (4) and the concentrations of theophylline, caffeine, theobromine, and paraxanthine were determined by ion-pair high-performance liquid chromatography (10), using a 30- × 0.39-cm (length × internal diameter) μBondapak C-18 (10 μm) column (Waters Inc., Milford, MA) with a 3-cm precolumn packed with Corasil C-18 (37–50 μm) (Waters Inc.). Detection limits were between 0.5 and 1 mg/liter. Statistical analysis was by t test or one-way analysis of variance followed by the Newman-Keuls test. The Kruskal-Wallis test followed by Duncan's nonparametric multiple comparison (11) was used in case of unequal variances.

Results

The serum concentrations of caffeine and some of its metabolites on the third and sixth day of twice daily caffeine injections are summarized in Table I. The concentrations of paraxanthine, theobromine, and theophylline each were approximately one-tenth that of caffeine. Sixteen and 24 hr after the last injection of caffeine (i.e., at the time of theophylline infusion), none of these compounds were detectable in serum. The body weight of the caffeine pretreated rats at that time was slightly but statistically significantly lower than that of the controls; only the controls had gained weight during the 7 days of injections (Table II).

Theophylline produced maximal seizures, i.e., flexion of the forelimbs and tonic extension of the hind limbs, usually leading to death shortly after onset of seizures. Pretreatment with caffeine was associated with a statistically significant increase in the brain and CSF concentrations of theophylline required to produce seizures (Table II).

The results of the paraxanthine-theophylline interaction study are summarized in Table III. The serum concentrations of paraxanthine produced by the 4-mg/kg injection exceeded the concentrations of this metabolite found during chronic caffeine administration (Table I). It should be noted however that the concentrations were determined in serum from blood samples obtained 10 min after paraxanthine injection and 1 hr after caffeine injection, respectively. Paraxanthine was also found in the CSF, in concentrations about one-half of those in serum. None of the paraxanthine doses had any apparent effect on the total dose and the serum and CSF concentrations of theophylline required to produce maximal seizures.

Discussion

Intravenous infusion of the methylxanthine drug theophylline at various rates until onset of maximal convulsions in rats has revealed that concentrations of the drug in the CSF at this pharmacologic end point are independent of infusion rate whereas concentrations in serum and whole brain increase with increasing infusion rate (4). This shows that theophylline in CSF, but not at the other sampling sites, is in rapid equilibrium with and therefore reflects the drug concentrations at the site of action. A similar experiment with caffeine showed increasing drug concentrations in CSF with decreasing infusion rate, indicative of rapid development of functional tolerance (2). The caffeine concentration in the CSF at onset of maximal seizures was 348 mg/liter upon rapid drug infusion and onset of effect in 11 min; it increased to 428 mg/liter upon slow drug

Table I. Concentrations of Caffeine and Metabolites in Serum of Rats during Repeated Caffeine Injections[a]

Compound	Day 3 (n = 22)	Day 6 (n = 21)
Caffeine	33.3 ± 6.3	39.2 ± 8.0
Paraxanthine	3.68 ± 1.03	3.36 ± 1.63
Theobromine	3.22 ± 1.40	3.38 ± 1.58
Theophylline	3.70 ± 1.06	3.45 ± 1.42

[a] Results are reported as mean ± SD and are expressed in mg/liter.

Table II. Effect of Preexposure to Repeated Caffeine Injections on Concentrations of Theophylline at Onset of Maximal Seizures in Rats Infused with Theophylline[a]

Variable	Controls	16 hr after caffeine	24 hr after caffeine
No. of animals	10	12	10
Body weight (g)[b]			
Before	223 ± 6	216 ± 8	217 ± 7
After	234 ± 7[c]	220 ± 14[d]	211 ± 19[d]
Infusion time (min)	33.0 ± 1.2	33.8 ± 2.2	30.7 ± 4.3
Total dose (mg/kg)	288 ± 6	314 ± 12[d, e]	296 ± 24
Serum concentration (mg/liter)	362 ± 55	400 ± 45	368 ± 78
Brain concentration (mg/kg)	227 ± 13	248 ± 14[f]	243 ± 22[f]
CSF concentration (mg/liter)	215 ± 13	235 ± 14[g]	239 ± 38[g]

[a] Results are reported as mean ± SD.
[b] Body weight immediately before and 1 day after 7 days of caffeine administration.
[c] Significant weight gain, $P < 0.05$ by paired t test.
[d] Significantly different from controls, $P < 0.01$ by Kruskal-Wallis test followed by Duncan's multiple comparison, $P < 0.05$.
[e] Significantly different from 24-hr group as in footnote *d*.
[f] Significantly different from controls, $P < 0.01$ by one-way analysis of variance followed by the Newman-Keuls test, $P < 0.05$.
[g] Significantly different from controls as in footnote *d*, except Kruskal-Wallis test, $P < 0.05$.

Table III. Effect of Acute Paraxanthine Administration on Concentrations of Theophylline at Onset of Maximal Seizures in Rats Infused with Theophylline[a]

Variable	Controls	Paraxanthine (mg/kg)		
		1	2	4
Body weight (g)	200 ± 8	199 ± 13	201 ± 11	198 ± 10
Serum paraxanthine (mg/liter)				
Before infusion[b]	—	1.68 ± 0.18	2.65 ± 0.22	5.04 ± 1.03
At seizure onset	—	1.19 ± 0.09	1.96 ± 0.57	4.18 ± 0.89
CSF paraxanthine (mg/liter)				
At seizure onset	—	0.64 ± 0.21	1.08 ± 0.15	2.05 ± 0.55
Theophylline				
Infusion time (min)	33.7 ± 1.7	34.0 ± 2.1	34.0 ± 0.9	33.8 ± 1.2
Total dose (mg/kg)	345 ± 12	350 ± 16	347 ± 20	348 ± 20
Serum concentration (mg/liter)	429 ± 17	431 ± 23	423 ± 16	435 ± 22
CSF concentration (mg/liter)	229 ± 25	239 ± 14	231 ± 23	229 ± 14

[a] Results are reported as mean ± SD, $n = 6$.
[b] Ten minutes after paraxanthine injection.

infusion and onset of effect in 82 min. This change of 23% within about 71 min of drug exposure reflects the rapid development of functional tolerance to the neurotoxic effect of caffeine. In comparison, repeated pretreatment with caffeine for 7 days caused an increase of only about 11% in the CSF concentration of theophylline required to produce maximal seizures in the present study. Finn and Holtzman (3) have reported that rats tolerant to stimulation of *locomotor* activity by caffeine were also tolerant to theophylline and a theophylline derivative, but not to any of six nonxanthine stimulants. The relative magnitude of this tolerance produced by daily caffeine ingestion in the drinking water was much more pronounced than what we observed with respect to *neurotoxicity* and persisted for more than 24 hr after the last dose.

It appears that locomotor stimulation by methylxanthines involves blockade of central adenosine receptors (9). Chronic exposure to caffeine or theophylline produces up-regulation of these receptors (12). According to some investigators, such up-regulation of A_1-adenosine receptors is accompanied by an elevation of seizure thresholds for certain convulsants (12) (methylxanthines were not tested as convulsants). On the other hand, there is increasing evidence that the convulsant effects of the methylxanthines are mediated by central benzodiazepine receptors (13, 14). It appears that the latter are less responsive to up-regulation by caffeine than are A_1-adenosine receptors.

Paraxanthine in low concentrations causes locomotor depression in mice (9). A possibility that this metabolite of caffeine is responsible for the apparent cross-tolerance to theophylline-induced convulsions produced by preexposure of the animals to caffeine is

not supported by our limited observations. In general, functional (cross-) tolerance development may not be a significant factor in individual susceptibility to the neurotoxicity of theophylline.

This research was supported in part by Grant GM 20852 from the National Institute of General Medical Sciences, National Institutes of Health.

1. Robertson D, Wade D, Workman R, Woosley RL, Oates JA. Tolerance to the humoral and hemodynamic effects of caffeine in man. J Clin Invest **67**:1111–1117, 1981.
2. Yasuhara M, Levy G. Rapid development of functional tolerance to caffeine-induced seizures in rats. Proc Soc Exp Biol Med **188**:185–190, 1988.
3. Finn IB, Holtzman SG. Pharmacologic specificity of tolerance to caffeine-induced stimulation of locomotor activity. Psychopharmacology **93**:428–434, 1987.
4. Ramzan IM, Levy G. Kinetics of drug action in disease states. XVI. Pharmacodynamics of theophylline-induced seizures in rats. J Pharmacol Exp Ther **236**:708–713, 1986.
5. Yasuhara M, Levy G. Caffeine is a potential risk factor for theophylline neurotoxicity. J Pharm Sci **77**:745–747, 1988.
6. Ramzan IM, Levy G. Chronic theophylline administration has no apparent effect of theophylline concentrations required to produce seizures in rats. Proc Soc Exp Biol Med **182**:176–180, 1986.
7. Arnaud MJ. Identification, kinetic and quantitative study of [2-^{14}C] and [1-Me-^{14}C]caffeine metabolites in rat's urine by chromatographic separations. Biochem Med **16**:67–76, 1976.
8. Bortolotti A, Jiritano L, Bonati M. Pharmacokinetics of paraxanthine, one of the primary metabolites of caffeine, in the rat. Drug Metab Dispos **13**:227–231, 1985.
9. Snyder SH, Katims JJ, Annau Z, Bruns RF, Daly JW. Adenosine receptors and behavioral actions of methylxanthines. Proc Natl Acad Sci USA **78**:3260–3264, 1981.
10. Lauff JJ. Ion-pair high-performance liquid chromatographic procedure for the quantitative analysis of theophylline in serum samples. J Chromatogr **417**:99–109, 1987.
11. Wall FJ. Statistical Data Analysis Handbook. New York: McGraw-Hill, pp 4.12–4.19, 1982.
12. Szot P, Sanders RC, Murray TF. Theophylline induced upregulation of A_1-adenosine receptors associated with reduced sensitivity to convulsants. Neuropharmacology **26**:1173–1180, 1987.
13. Vellucci SV, Webster RA. Antagonism of caffeine-induced seizures in mice by Ro15-1788. Eur J Pharmacol **97**:289–293, 1984.
14. Seale TW, Carney JM, Rennert OM, Flux M, Skolnick P. Coincidence of seizure susceptibility to caffeine and to the benzodiazepine inverse agonist, DMCM, in SWR and CBA inbred mice. Pharmacol Biochem Behav **26**:381–387, 1987.