Sympathomimetic effects of paraxanthine and caffeine in humans

Caffeine is metabolized extensively (on average 80%) to paraxanthine. With regular caffeine consumption, average serum levels of paraxanthine are two thirds those of caffeine. Both caffeine and paraxanthine competitively and nonselectively inhibit adenosine receptors in vitro. To examine the contribution of paraxanthine to the pharmacologic activity of caffeine, we administered to 12 subjects in a crossover design oral caffeine (2 or 4 mg/kg) versus placebo or oral paraxanthine (same dose as caffeine) versus placebo, each after 3 days of methylxanthine abstinence. Both caffeine and paraxanthine significantly increased diastolic blood pressure, plasma epinephrine levels, and free fatty acids. Caffeine and paraxanthine produced a similar magnitude of response at 4 mg/kg; however, caffeine appeared to produce greater responses than paraxanthine at 2 mg/kg. Caffeine and paraxanthine have similar sympathomimetic actions. The activity of paraxanthine needs to be considered in understanding the clinical pharmacology of caffeine, particularly with chronic, repetitive caffeine consumption. (CLIN PHARMACOL THER 1995;58:684-91.)

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Caffeine is probably the most widely consumed stimulant in the world. Caffeine is metabolized extensively, primarily by demethylation to dimethylxanthines. On average, 80% of caffeine is metabolized to paraxanthine (1,7-dimethylxanthine), with smaller percentages metabolized to theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine) (see Structure).[1] With repetitive caffeine consumption, such as with regular coffee drinking, steady-state plasma paraxanthine levels reach about two thirds those of caffeine.[2] If paraxanthine has pharmacologic activity, it could contribute substantially to the effects of caffeine.

Caffeine acts primarily by competitively and nonselectively antagonizing the effects of adenosine on adenosine receptors.[3-5] The resultant effects in humans include mental stimulation, systemic catecholamine release, and sympathetic neural stimulation, including an increase in blood pressure and lipolysis with an increase in plasma free fatty acid concentrations.

That paraxanthine might have pharmacologic activity is suggested by evidence that paraxanthine is an equipotent adenosine antagonist to caffeine in vitro.[6] In addition, paraxanthine may have dopaminergic action, acting on brain D-1 receptors.[7,8] In rats the threshold doses of caffeine and paraxanthine to produce locomotor stimulation, which may be related to effects on dopaminergic neurons, are generally similar.[6,9] To the best of our knowledge, the pharmacologic effects of paraxanthine in humans have not been reported. The objectives of this study were to determine the effects of paraxanthine in humans and to compare the effects of paraxanthine with those of caffeine.

METHODS

Subjects. Twelve healthy volunteers, six men and six women, 19 to 49 years of age (mean age, 32 years), who were regular coffee drinkers and non-

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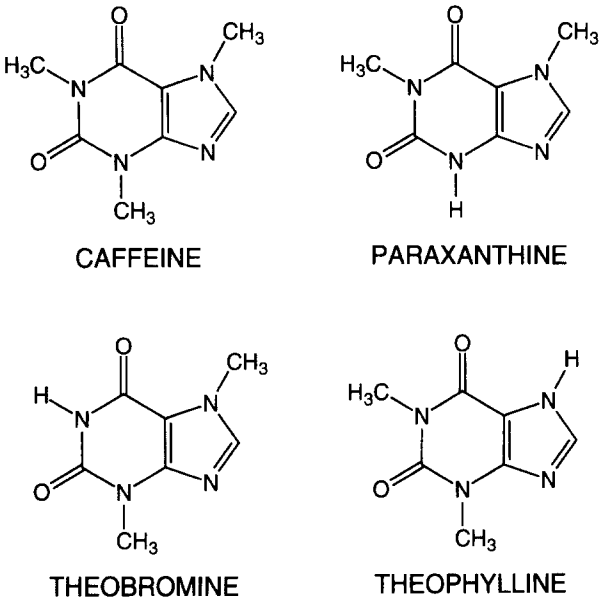

Chemical structures of caffeine and its major proximate metabolites.

smokers were recruited. Subjects were recruited from the medical center and newspaper advertisements. Estimated daily caffeine consumption ranged from 68 to 1168 mg (mean, 506 ± 324 mg). Daily caffeine intake was estimated on the basis of the self-reported typical daily consumption of various caffeine-containing beverages and food (i.e., coffee, tea, caffeinated sodas, and chocolate) and published caffeine contents for these various foods per ounce.[10-12] Plasma caffeine concentration on admission ranged from 0.06 to 4.6 mg/L (mean, 1.7 ± 1.5 mg/L). Each subject had a normal physical examination, electrocardiogram, and screening blood chemistry results. Subjects were not taking medications, including oral contraceptives. Written consent was obtained from each subject. The study was approved by the Committee on Human Research at the University of California, San Francisco.

Experimental protocol. The study was conducted in two separate phases, one with caffeine and the other with paraxanthine dosing, each lasting 8 days. The phases of the study were separated by at least 3 weeks. The sequence of phases was balanced.

Each study phase began with 3 days of abstinence from caffeine. Abstinence from alcohol was required for the duration of the study. On the afternoon of the third day, subjects were admitted to the General Clinical Research Center at San Francisco General Hospital, where they remained through the end of day 5. On day 4, after an overnight fast, subjects received a placebo capsule at 8 AM. On day 5 at 8 AM, subjects received a capsule containing either caffeine or paraxanthine in a dose of 4 mg/kg (n = 7) or 2 mg/kg (n = 5). Each subject received the same dose of caffeine and paraxanthine in the two phases of the study. Placebos were always given on day 4 because levels of caffeine and paraxanthine may persist at low levels for greater than 24 hours. Subjects were not aware of the order of placebo versus active drug or caffeine versus paraxanthine treatments. Days 6 through 8 involved repeated dosing as part of a caffeine metabolism study, which will be reported elsewhere.

On days 4 and 5 of each phase, intravenous catheters were placed in each forearm. Subjects received a capsule containing placebo, caffeine, or paraxanthine at 8 AM, as noted above. At 8:10 AM, each subject received a 5-minute intravenous infusion of 25 mg stable isotope-labeled caffeine (2-^{13}C,1,3-$^{15}N_2$ caffeine) as part of a caffeine metabolism study, which will be reported elsewhere. The 25 mg dose of caffeine is extremely small and was expected to have no pharmacologic effect. Blood samples were taken for measurement of plasma norepinephrine and epinephrine, free fatty acid, cortisol, caffeine, and paraxanthine concentrations at −30, −15, 0, and 30 minutes and 1, 1½, 2, and 3 hours from the time of ingestion of the capsule. Blood pressure and heart rate were measured every 15 minutes with an automatic blood pressure recording device (Dynamap, Critikon, Inc., Raritan, N.J.). Subjects were kept fasting and supine for the duration of the 3-hour study.

Caffeine, USP, was obtained from Fisher Scientific Co. (Chemical Mfg. Division, Fair Lawn, N.J.). Paraxanthine (Sigma Chemical Co., St. Louis, Mo.) was crystallized from distilled water and dried under vacuum. Gelatin capsules (No. 2; Eli Lily, Indianapolis, Ind.) were filled with the appropriate dose of caffeine or paraxanthine or lactose (placebo) in the investigator's laboratory.

Analytic chemistry. Plasma concentrations of norepinephrine and epinephrine were measured by HPLC with electrochemical detection.[13] Plasma concentrations of nonesterified fatty acids were measured by an enzymatic colorimetric method with kits obtained from Wako Chemicals USA, Inc. (Dallas, Texas). Plasma cortisol levels were measured by a competitive binding technique.[14] Plasma concentrations of caffeine and paraxanthine were measured by HPLC.[2]

Data analysis. Response data were expressed as peak change from baseline and area under the re-

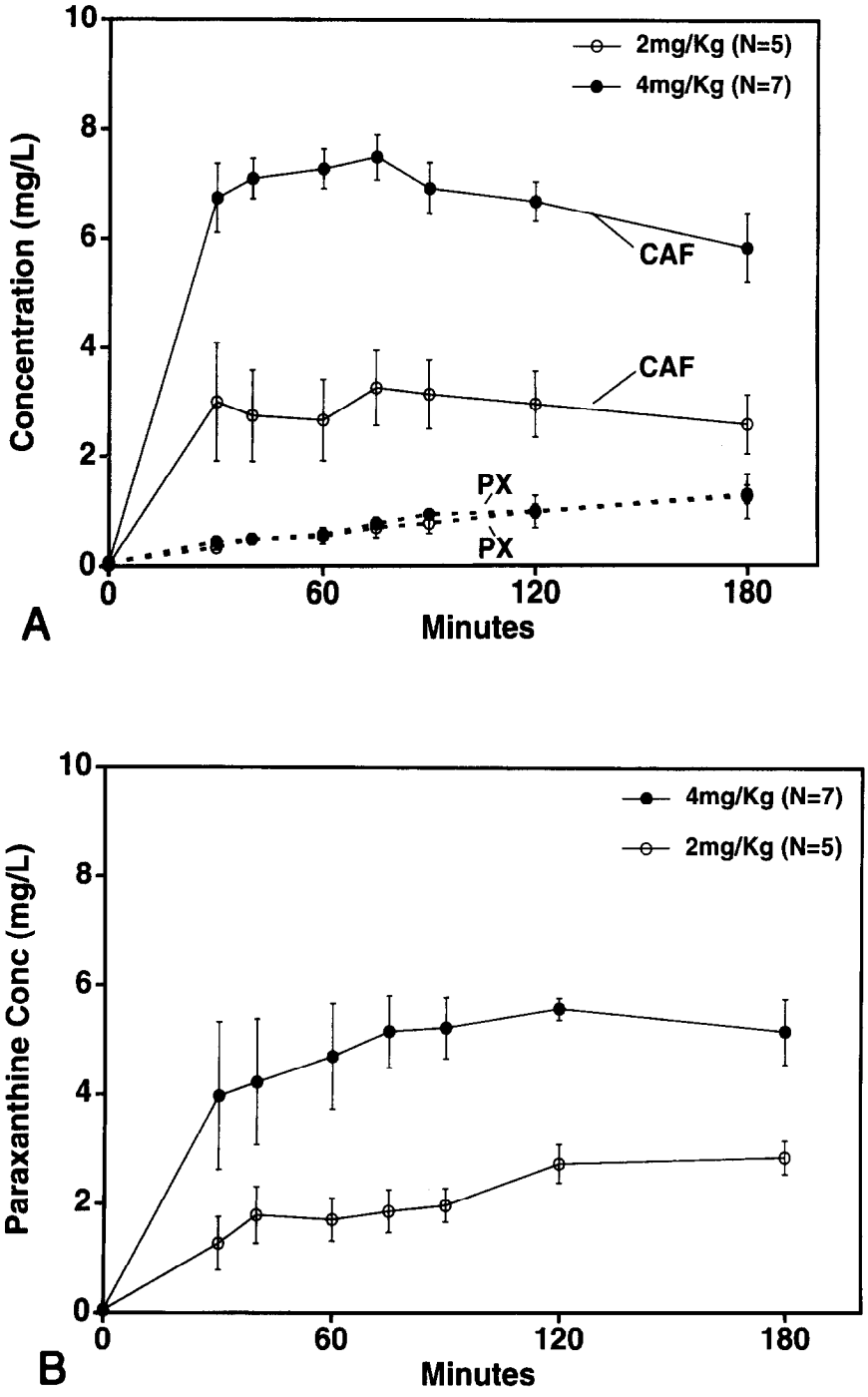

Fig. 1. A, Plasma concentrations of caffeine (CAF; *solid lines*) and paraxanthine (PX; *dashed lines*) after oral doses of caffeine. **B,** Plasma concentrations (conc) of paraxanthine after oral doses of paraxanthine. *Bars* indicate SEM.

sponse-time curve from 0 to 180 minutes. The area under the response-time curve represents the integrated response over time and is taken as the primary dependent variable. The peak change is described primarily to show the magnitude of response in a more familiar dimension. Comparisons were made by analysis of variance, comparing placebo versus active drug at two dose levels. The Tukey posttest was used for multiple comparisons.[15] Significant differences indicate $p < 0.05$.

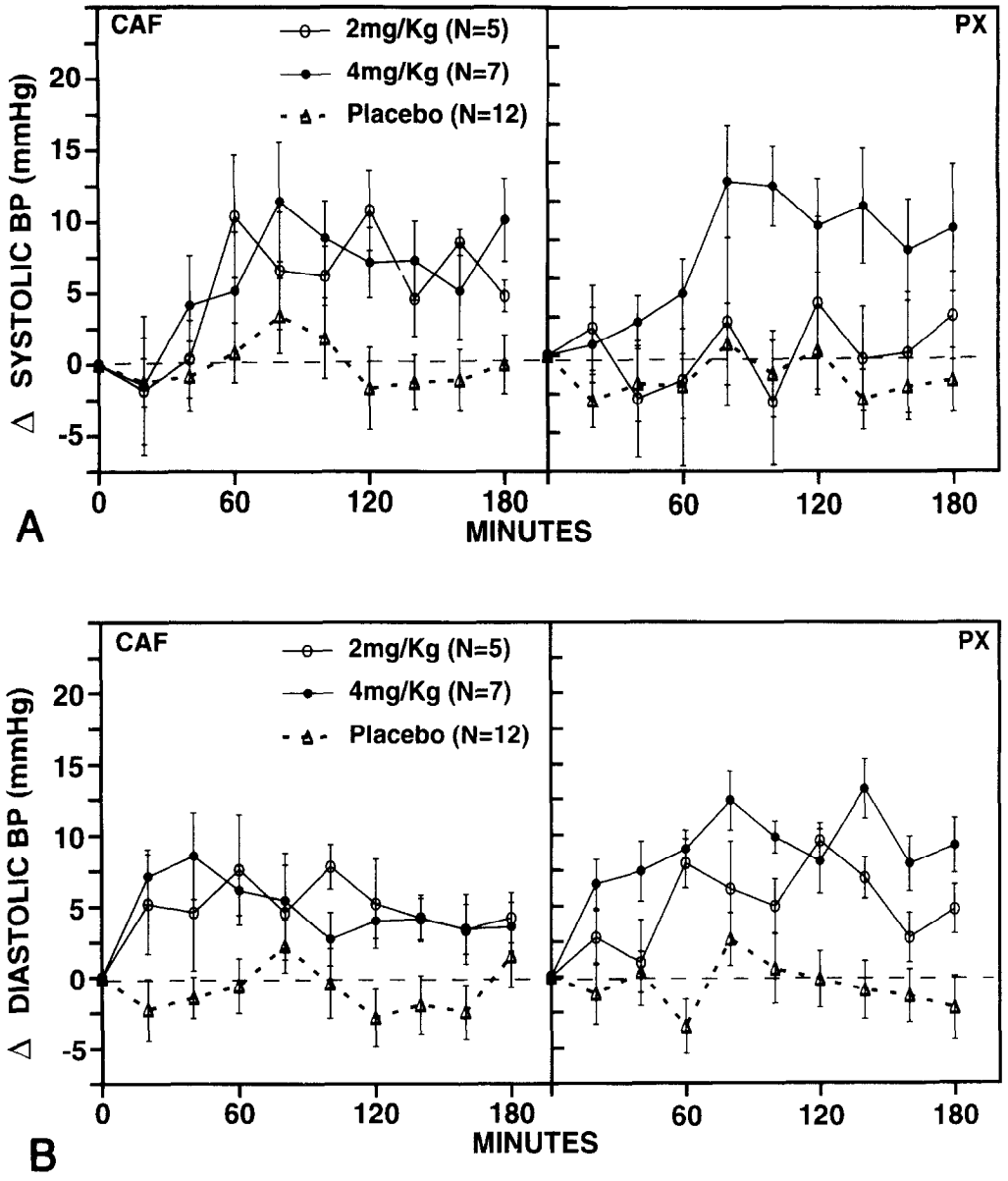

Fig. 2. Systolic blood pressure (BP) response (change from baseline) (**A**) and diastolic blood pressure response (**B**) after oral doses of caffeine (CAF), paraxanthine (PX), and placebo.

RESULTS

Plasma caffeine and paraxanthine levels. The average plasma concentrations of caffeine before dosing with caffeine or paraxanthine were 0.04 ± 0.04 mg/L and 0.08 ± 0.08 mg/L, respectively. The average plasma concentrations of paraxanthine before dosing with caffeine or paraxanthine were 0.06 mg/L ± 0.05 mg/L and 0.06 ± 0.05 mg/L, respectively. These low concentrations indicate that subjects complied with instructions not to consume caffeinated beverages before the study. Average plasma concentrations after caffeine and paraxanthine dosing are shown in Fig. 1. After caffeine dosing, plasma caffeine levels peaked at an average concentration of 3.2 ± 1.5 mg/L 75 minutes after 2 mg/kg and 7.5 ± 1.0 mg/L 75 minutes after 4 mg/kg doses. After caffeine dosing, plasma paraxanthine levels peaked at 1.4 ± 0.9 mg/L 300 minutes and 1.6 ± 0.3 mg/L 540 minutes after 2 mg/kg and 4 mg/kg doses, respectively. After paraxanthine dosing, the paraxanthine concentration peaked at

Table I. Cardiovascular and metabolic effects of caffeine and paraxanthine

	Systolic blood pressure (mm Hg)			*Diastolic blood pressure (mm Hg)*			*Heart rate (min^{-1})*		
	Baseline	*Maximum change*	*AUC*	*Baseline*	*Maximum change*	*AUC*	*Baseline*	*Maximum change*	*AUC*
Caffeine									
2 mg/kg (n = 5)	116 ± 14	16 ± 8	1,015 ± 756	70 ± 11	13 ± 5	928 ± 822*	70 ± 12	8 ± 6	−67 ± 1238
4 mg/kg (n = 7)	114 ± 7	16 ± 10	1,104 ± 1,132	70 ± 4	13 ± 6	904 ± 679*	60 ± 6	6 ± 3	−40 ± 299
Placebo (n = 12)	116 ± 9	10 ± 7	50 ± 952	74 ± 11	6 ± 5	−134 ± 876	63 ± 11	7 ± 7	115 ± 926
Paraxanthine									
2 mg/kg (n = 5)	117 ± 14	10 ± 8	77 ± 1,412	67 ± 15	13 ± 4	930 ± 715	70 ± 12	5 ± 4	−474 ± 468
4 mg/kg (n = 7)	114 ± 6	16 ± 9*	1,178 ± 1,014	68 ± 9	16 ± 5*	1,636 ± 801*	61 ± 13	5 ± 5	−304 ± 597
Placebo (n = 12)	117 ± 11	7 ± 5	−192 ± 808	71 ± 10	8 ± 5	−49 ± 772	60 ± 9	9 ± 7	268 ± 758

AUC, Area under the response-time curve from 0 to 180 minutes.
*$p < 0.05$ for drug at a particular dose compared with placebo.
†$p < 0.05$ for 2 mg compared with 4 mg dose.

2.8 ± 0.7 mg/L 180 minutes and 5.6 ± 0.6 mg/L 120 minutes after 2 mg/kg and 4 mg/kg doses, respectively.

Cardiovascular responses. No effects were observed after placebo dosing, supporting the assumption that the 25 mg intravenous dose of labeled caffeine does not produce significant cardiovascular effects. Both caffeine and paraxanthine decreased the area under the heart rate time-response curve in relation to placebo, but the effect was not significant. At 4 mg/kg, both caffeine and paraxanthine increased systolic and diastolic blood pressure (Table I; Fig. 2). The increases in systolic blood pressure, although of considerable magnitude, were not statistically significant, probably because of marked intersubject variability. At 2 mg/kg, caffeine increased systolic and diastolic blood pressure to the same extent as at 4 mg/kg. However, at 2 mg/kg, paraxanthine had no effect on systolic blood pressure but did significantly increase diastolic blood pressure. At 4 mg/kg, paraxanthine increased systolic and diastolic blood pressure to an extent similar to that of caffeine at the same dose.

Hormonal and metabolic responses. Both caffeine and paraxanthine increased plasma concentrations of catecholamines. Although both epinephrine and norepinephrine levels increased, only epinephrine responses were significantly different from placebo responses (Table I; Fig. 3). Norepinephrine responses were on average substantial but were also extremely variable. Plasma epinephrine responses were dose related. Caffeine and paraxanthine had similar effects on epinephrine at 4 mg/kg, whereas the effects of caffeine were greater than those of paraxanthine at 2 mg/kg. Plasma free fatty acid concentrations, reflecting catecholamine-induced lipolysis, were increased by both caffeine and paraxanthine (Table I; Fig. 4). At 4 mg/kg, responses to caffeine and paraxanthine were similar. At 2 mg/kg, caffeine produced a response greater than that of paraxanthine. No significant effects of caffeine or paraxanthine on serum cortisol levels were seen.

DISCUSSION

Caffeine is well known to have sympathomimetic effects in people.[16-18] We have demonstrated for the first time that paraxanthine, the major metabolite of caffeine, has similar pharmacologic activity in people. We found that paraxanthine activated the sympathetic nervous system, resulting in catecholamine release, increased blood pressure, and enhanced lipolysis. Paraxanthine produced a magnitude of cardiovascular, hormonal, and metabolic effects similar to that of caffeine, although the dose-response characteristics for caffeine and paraxanthine appeared to be different. Both had similar effects at higher doses (4 mg/kg), but in general caffeine was more potent than paraxanthine at lower doses (2 mg/kg).

Several methodologic issues warrant mention. First, the subjects were regular coffee consumers who were abstinent from caffeine for 3 days before the study.

Plasma epinephrine (pg/ml)			*Plasma norepinephrine (pg/ml)*			*Plasma free fatty acids (mEq/L)*		
Baseline	*Maximum change*	*AUC*	*Baseline*	*Maximum change*	*AUC*	*Baseline*	*Maximum change*	*AUC*
30 ± 17	35 ± 19*	3,060 ± 1,975*	221 ± 98	61 ± 45	629 ± 4,127	0.439 ± 0.223	0.339 ± 0.094*	38.0 ± 10.6*
27 ± 10	63 ± 18*†	6,981 ± 2,300*†	198 ± 66	182 ± 98*	12,596 ± 8,788	0.269 ± 0.094	0.425 ± 0.101*	50.4 ± 11.9*
29 ± 20	18 ± 15	392 ± 2113	224 ± 99	59 ± 76	−261 ± 10,624	0.404 ± 0.182	0.192 ± 0.097	13.0 ± 13.0
24 ± 18	27 ± 21	1,745 ± 2,757	186 ± 76	310 ± 459	12,456 ± 15,822	0.366 ± 0.123	0.203 ± 0.127	14.2 ± 13.5
19 ± 8	68 ± 18*†	5,975 ± 794*†	236 ± 96	161 ± 115	10,296 ± 6,706	0.283 ± 0.093*	0.352 ± 0.220	41.0 ± 28.5*
19 ± 12	19 ± 15	1,506 ± 1,499	211 ± 71	59 ± 60	47 ± 13,094	0.465 ± 0.213	0.154 ± 0.144	5.7 ± 14.7

Abstinence was confirmed by low plasma caffeine concentrations before the test capsules. That tolerance develops quickly to the effects of methylxanthines is well described in the literature.[16-19] Therefore there is concern that the effects of caffeine and paraxanthine that we measured might be influenced by previous tolerance as a result of habitual caffeine consumption. However, this is unlikely to be a confounder because tolerance dissipates rapidly after discontinuation of caffeine consumption, and resensitization to caffeine effects appears to be complete by 3 days.[17,18] In addition, because caffeine and paraxanthine were administered after the same duration of caffeine abstinence, any tolerance to the actions of methylxanthine would be equivalent for the two treatments.

Another methodologic issue is the possible confounding effect of the 25 mg intravenous labeled caffeine dose that was administered for the purpose of studying caffeine kinetics. This tracer dose of caffeine resulted in low plasma levels of caffeine that could have added to the measured effects of caffeine and paraxanthine. However, these levels of caffeine were quite low compared with those produced by the oral doses of caffeine and paraxanthine, and no responses were seen after placebo capsule dosing, when labeled caffeine was also given. Thus we conclude that the contribution of the low dose of intravenous caffeine to the pharmacologic effects we observed was insignificant and does not affect the validity of our conclusions.

Finally, the sequence of treatments was always placebo first and then caffeine or paraxanthine. It is generally desirable to balance the sequence of placebo versus active drug, but in this case we chose not to because of possible carryover of concentrations of caffeine, paraxanthine, or their metabolites from day 1 to day 2. Although we cannot exclude some order effect between placebo and caffeine or paraxanthine, we can be sure that for the purposes of comparisons of caffeine versus paraxanthine, both compared with placebo treatment, our results are valid.

Our results are consistent with those of adenosine receptor binding studies and rodent locomotion studies showing that paraxanthine and caffeine have similar effects. Most binding studies and some locomotion studies indicate that paraxanthine is more potent than caffeine, although the potency may vary with the particular tissue or rodent strain.[4-6,9] However, in our human data it appears that there is a different dose response for caffeine and paraxanthine, suggesting that caffeine is more potent than paraxanthine in the lower part of the dose-response curve. Thus caffeine produced similar responses at 2 and 4 mg/kg, whereas paraxanthine generally produced the same effects as caffeine at 4 mg/kg but a lesser effect at 2 mg/kg. Assuming that the affinity of caffeine and paraxanthine for adenosine receptors in humans is similar to one another, as is the case in vitro,[6] a pharmacokinetic explanation for different dose-response relationships must be entertained. Caffeine is less polar and permeates

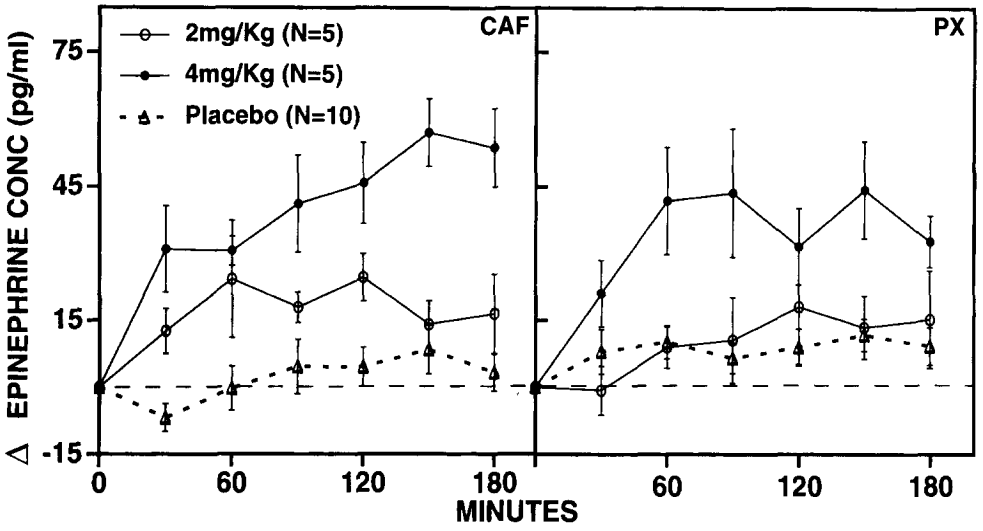

Fig. 3. Plasma epinephrine response to oral doses of caffeine (CAF) and paraxanthine (PX).

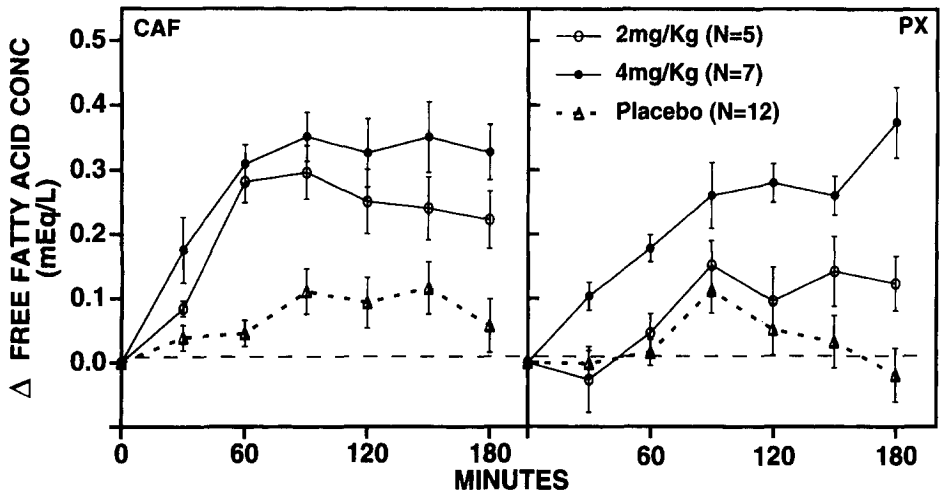

Fig. 4. Plasma free fatty acid responses to oral doses of caffeine (CAF) and paraxanthine (PX).

cells more readily than does paraxanthine, which is more polar. For example, in rats the brain/blood partition ratio for caffeine is much greater than that for paraxanthine.[7] Thus higher concentrations of caffeine compared with paraxanthine at sites of action could explain the apparent greater potency of caffeine at lower concentrations.

The implications of our study with respect to the human pharmacology of caffeine are as follows. After single doses of caffeine, our study suggests that paraxanthine levels are relatively low (Fig. 1). At those concentrations, paraxanthine does not contribute much to the effect of the parent caffeine. However, with long-term caffeine exposure, there is accumulation of paraxanthine to substantial levels.[2] On average, paraxanthine levels are two thirds those of caffeine. Thus with long-term dosing, paraxanthine almost certainly contributes to the pharmacologic activity of caffeine. Long-term dosing of caffeine is associated with nearly complete development of tolerance to most of the effects of caffeine, although some effects may persist.[16] The receptor adaptation that occurs as a part of the development of tolerance would have to occur to the combined effects of caffeine and paraxanthine. Thus paraxanthine is expected to contribute to the development of tolerance and caffeine-withdrawal symptoms. These issues remain to be studied.

There is likely to be considerable individual variation in the extent of conversion of caffeine to paraxanthine. If so, because paraxanthine has pharmacologic

activity, the extent of conversion of caffeine to paraxanthine would be a factor in determining individual differences in pharmacologic responses to caffeine.

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References

1. Lelo A, Miners JO, Robson R, Birkett DJ. Quantitative assessment of caffeine partial clearances in man. Br J Clin Pharmacol 1986;22:183-6.
2. Denaro CP, Brown CR, Wilson M, Jacob P III, Benowitz NL. Dose-dependency of caffeine metabolism with repeated dosing. CLIN PHARMACOL THER 1990;48:277-85.
3. Biaggioni I, Paul S, Puckett A, Arzubiaga C. Caffeine and theophylline as adenosine receptor antagonists in humans. J Pharmacol Exp Ther 1991;258:588-93.
4. Daly JW, Butts-Lamb P, Padgett W. Subclasses of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines. Cell Mol Neurobiol 1983;3:69-80.
5. Schwabe U, Ukena D, Lohse MJ. Xanthine derivatives as antagonists at A_1 and A_2 adenosine receptors. Naunyn Schmiedebergs Arch Pharmacol 1985;330:212-21.
6. Snyder SH, Katims JJ, Annau Z, Bruns RF, Daly JW. Adenosine receptors and behavioral actions of methylxanthines. Proc Natl Acad Sci USA 1981;78:3260-4.
7. Ferre S, Guix T, Salles J, et al. Paraxanthine displaces the binding of [^{3}H] SCH 23390 from rat striatal membranes. Eur J Pharmacol 1990;179:295-9.
8. Ferre S, Herrera-Marschitz M, Grabowska-Anden M, Casas M, Ungerstedt U, Anden NE. Postsynaptic dopamine/adenosine interaction: II: postsynaptic dopamine agonism and adenosine antagonism of methylxanthines in short-term reserpinized mice. Eur J Pharmacol 1991; 192:31-7.
9. Logan L, Seale TW, Carney JM. Inherent differences in sensitivity to methylxanthines among inbred mice. Pharmacol Biochem Behav 1986;24:1281-6.
10. Blauch JL, Tarka SM Jr. HPLC determination of caffeine and theobromine in coffee, tea, and instant hot cocoa mixes. J Food Sci 1983;48:745-50.
11. Galasko GTF, Furman KI, Alberts E. The caffeine contents of non-alcoholic beverages. Food Chem Toxicol 1989;27:49-51.
12. Stavric B, Klassen R, Watkinson B, Karpinski K, Stapley R, Fried P. Variability in caffeine consumption from coffee and tea: possible significance for epidemiological studies. Food Chem Toxicol 1988;26:111-8.
13. Anton AH, Sayre DF. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 1962; 138:360-75.
14. Murphy BEP. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding assay. J Clin Endocrinol 1967;27:975-90.
15. Neter J, Wasserman W. Applied linear statistical models. Homewood, Illinois: Richard D. Irwin, 1974:474-7.
16. Denaro CP, Brown CR, Jacob P III, Benowitz NL. Effects of caffeine with repeated dosing. Eur J Pharmacol 1991;40:273-8.
17. Robertson D, Wade D, Workman R, Woosley RL, Oates JA. Tolerance to the humoral and hemodynamic effects of caffeine in man. J Clin Invest 1981;67:1111-7.
18. Shi J, Benowitz NL, Denaro CP, Sheiner LB. Pharmacokinetic-pharmacodynamic modeling of caffeine: tolerance to pressor effects. CLIN PHARMACOL THER 1993;53:6-14.
19. Evans SM, Griffith RR. Caffeine tolerance and choice in humans. Psychopharmacology 1992;108:51-9.