Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 2, pp. 245–252, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00306-8

# Oral and IP Caffeine Pharmacokinetics Under a Chronic Food-Limitation Condition

CHYAN E. LAU,[1] FANG MA AND JOHN L. FALK

*Department of Psychology–Busch, Rutgers University, New Brunswick, NJ 08903*

Received 6 June 1994

LAU, C. E., F. MA AND J. L. FALK. *Oral and IP caffeine pharmacokinetics under a chronic food-limitation condition.* PHARMACOL BIOCHEM BEHAV **50**(2) 245–252, 1995.—For food-limited rats, serum caffeine was proportional to IP caffeine doses (10–40 mg/kg) for $C_{max}$ and area under the curve [$AUC_{(0\text{-}24\ h)}$], whereas the three dimethylxanthine (DMX) metabolites of caffeine were disproportional over the dose range. Steady-state concentrations of caffeine and the three metabolites were evident at the 11th day of chronic, daily caffeine IP 20 mg/kg doses. Both caffeine and the three metabolites were dose proportional for $C_{max}$ and $AUC_{(0\text{-}24\ h)}$ by schedule-induced oral caffeine self-administration within the dose range taken (9–38 mg/kg). These results contrast with the nonlinear kinetics of caffeine reported for rats under ad lib conditions. Elimination rate constants ($K_{el}$) remained the same for the two routes, but apparent volume of distribution ($AV_d$) and clearance (Cl) were different. The order of the $K_{el}$ values was caffeine > paraxanthine > theophylline > theobromine. The effects of linear vs. nonlinear caffeine pharmacokinetics may have distinct implications for the resulting pharmacodynamics.

Caffeine pharmacokinetics  Food deprivation  Oral drug  Schedule-induced polydipsia  Dimethylxanthines

CAFFEINE (1,3,7-trimethylxanthine) is the psychoactive agent most widely self-administered by humans (17). It has pharmacologic effects as a central nervous stimulant, cardiotonic, and diuretic (32,34). Caffeine is biotransformed to dimethylxanthines (DMXs), theobromine, paraxanthine, theophylline, and other metabolites through several enzymatic pathways. However, the P-450 cytochrome oxygenases play a major role in the primary metabolism of caffeine (1,30). Dose-dependent or nonlinear kinetics have been reported in rats (1,23,25) and humans (11,39), which suggests a saturation of metabolic transformations. Caffeine also exhibits dose-independent or linear kinetics at normal human intake levels (7,33). In animals, to study the effects of caffeine on behavior, a food-deprivation regimen is often used to implement a food-reinforced behavioral baseline. In humans, food deprivation or restriction can occur for cosmetic, health, or economic reasons. To best of our knowledge, almost all pharmacokinetic studies of caffeine have used free-feeding animals. Food restriction for 28 days in male rats enhanced hexobarbital metabolism by increasing the hepatic microsomal metabolizing enzymes (35). One of the aims of the present study was to investigate both acute and chronic caffeine pharmacokinetics by the IP route in food-limited rats, which were held to 80% of their free-feeding, adult weights by limiting food rations.

Oral self-administration is the most common route by which humans receive caffeine. The consumption pattern of caffeine-containing beverages and foods may occur at one sitting or be spread throughout the day to yield approximately equal intakes (36). Different consumption patterns may result in different serum methylxanthine profiles. These different profiles are considered in the present study because they may account for differences in long-term effects, such as the rate of development of tolerance (29). Caffeine absorption from the gastrointestinal tract is rapid and complete (7). For oral and intravenous doses, plasma concentration curves were superimposable after an initial absorption phase, which suggests that there is no important hepatic first-pass effect in humans (3, 5) or animals (1). A schedule-induced drinking procedure (13) was used that permitted oral-self administration of caffeine solutions to evaluate the pharmacokinetics of caffeine after daily, 3-h sessions of caffeine drinking, and the results were compared with those from the IP route. These two routes differ in the rate of exposure to the drug. Also, for the IP route the drug is imposed by the experimenter, whereas with schedule induction the drug is self-administered. Inasmuch as the three DMX metabolites of caffeine possess intrinsic pharmacologic activity (19,37,38) and contribute to the pharmacodynamic effects of caffeine, their profiles were studied concurrently.

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

## METHODS

### *Drug*

Caffeine was purchased from Sigma Chemical Co. (St. Louis, MO). For the IP route experiments, caffeine was dissolved in sodium benzoate (37.5 mg/ml) solution and administered in an injection volume of 1 ml/kg body wt. Doses were calculated as the base.

### *Reagents, Serum Sampling, and High Performance Liquid Chromatography*

Theobromine, paraxanthine, and theophylline were purchased from Sigma Chemical Co. Other reagents were obtained from standard commercial sources. The serum sampling procedure and a sensitive serum microsample (10–50 μl) high performance liquid chromatography method used for the determination of caffeine and its metabolites have been described previously (26–28). Briefly, 50 μl of caffeine and the three DMX metabolite working standards or serum sample and 25 μl of internal standard, β-hydroxyethyltheophylline (2 μg/ml), were mixed with 1 M borate buffer pH 9.0 (100 μl) in a 15-ml conical centrifuge tube. Then, 1 ml of chloroform : ethanol (82.5 : 17.5) was added, and the result was mixed and centrifuged. The organic layer was evaporated to dryness and resuspended in 50 μl of mobile phase for separation on an Altex (San Ramon, CA) Ultrasphere C18 column (5-μm particle size, 150 × 2.0 mm i.d). Absorbance at 270 nm was monitored on a Perkin-Elmer (Norwalk, CT) integrator LCI-100. The mobile phase was methanol–1% acetic acid–28 mM sodium acetate buffer containing 1.4 mM tetrabutylammonium phosphate adjusted to pH 2.15 with 40% phosphoric acid (12 : 52 : 36, vol./vol./vol.). The flow rate was set at 0.3 ml/min and operated at a pressure of 103 bar (1500 p.s.i.).

### *Animals*

Eight adult male, albino rats of the Holtzman strain (Madison, WI) with a mean initial body weight of 385 g (range 383–391) were housed individually in stainless-steel cages in a temperature-regulated room with a 12-h light–dark cycle (lights on at 0700–1900 h).

### *Caffeine Oral Self-Administration by a Schedule-Induction Method*

Animals were given a daily 3-h experimental session in individual Plexiglas chambers (30 × 26 × 23 cm) as described previously (15). Each chamber was equipped with a stainless-steel food pellet receptacle and a drinking fluid reservoir that consisted of a stainless-steel ball-bearing spout attached to a Nalgene graduated cylinder. Fluid intakes were recorded to the nearest milliliter. Animals were weighed at the same time each day, a fluid cylinder was attached to the chamber, and for the next 3 h a 45-mg food pellet (Bio Serv, Frenchtown, NJ) was delivered automatically into each food receptacle every 60 s (an FT 1-min schedule). Under this sort of food-delivery schedule, polydipsia occurs during each session (14). In the present experiment, the food-delivery schedule induced the oral self-administration of high volumes of caffeine solutions during each daily 3-h session.

### *Procedure*

After establishing ad lib weights, animals were reduced to 80% body wt. by limiting daily food rations, and held at these weights for the duration of the experiment. These experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). After weights were stabilized for 4 weeks, four animals received three acute IP caffeine doses in an ascending order: 10, 20, and 40 mg/kg. At least 4 days separated injections. Blood samples (100 μl) were collected at 0.33, 2, 3, 4, 8, 11, 24, and 31 h. Ten days after the last acute caffeine dose, daily 20 mg/kg caffeine IP doses were administered to animals for 21 days. Blood samples were taken on the 11th and 21st day at the same time intervals as in the acute phase, except samples at 31 h were not taken for the 11th day.

The second group of animals ($n = 4$) was used to study the serum caffeine concentration profiles under the schedule-induced oral self-administration procedure. Animals were given daily 3-h drinking sessions as described earlier. Initially, the session fluid available was distilled water (10 days), and then the self-administered dose of caffeine was sequentially increased by altering the drinking-solution solutes after several sessions at each level. Hourly intakes were recorded for each session, blood samples (100 μl) were drawn immediately after selected sessions (i.e., after 3 h of drinking a caffeine solution), and again at hours 4, 5, 6, 7, 11, 15, 19, and 24 after the start of the drinking session. Blood samples also were obtained at 1 and 2 h during a session, providing caffeine intakes remained as usual for the 2nd-h point (i.e., not disrupted by the sampling procedure); otherwise, samples at some time points were obtained during sessions on different days. The number of sessions for which a particular caffeine solution was offered, and the blood sampling sessions, were: 0.05 mg/ml caffeine (8 days, sampled on 6th day), 0.1 mg/ml caffeine (8 days, sampled on 6th day), 0.1 mg/ml caffeine in compound-solution vehicle [0.08% sodium saccharin and 1.5% glucose (9 days, sampled on 6th day)].

### *Data Analysis*

Elimination rate constants ($K_{el}$) were computed by log-linear regression analysis with assumption of a first-order, one-compartment, open model. The area under the curve was calculated by the trapezoidal method. Because $K_{el}$ values of DMX metabolites could not be calculated precisely using the present sampling schedules, $AUC_{(0-\infty)}$s only were calculated for caffeine and $AUC_{(0-24\ h)}$s were calculated for both caffeine and the DMX metabolites. The values reported as the maximum concentration ($C_{max}$) are the actual observed values for the sampling series. The bioavailability of the caffeine dose was considered to be equal to 1.0 at the dose levels used, as complete absorption of caffeine has been reported (7). Thus, the clearance ($Cl_{IP}$ or $Cl_{po}$) of caffeine was calculated as the dose divided by the $AUC_{(0-\infty)}$. The apparent volume of distribution ($AV_d$) was calculated as $Cl_{IP}$ or $Cl_{po}$ divided by $K_{el}$. Neither Cls nor $AV_d$s were calculated for the DMX metabolites.

Statistical analyses of the serum concentration-time profile data were performed by repeated-measures, one-way ANOVA with treatment as the factor within subjects. ANOVA also was performed with repeated measures, one between and one within, for between-group comparisons. Appropriate paired *t*-tests also were calculated.

## RESULTS

### *Acute IP Caffeine*

Serum concentration-time profiles and the kinetic parameters of caffeine and its metabolites, theobromine, paraxan-

thine, and theophylline at three acute caffeine doses (10, 20, and 40 mg/kg) are shown in Fig. 1 and Table 1. Serum caffeine concentration peaked at the 3rd h for the three caffeine doses. Formation of the three metabolites reached a maximum at the 8th h for the lowest dose and at the 11th h with larger caffeine doses under the sampling schedules. The $K_{el}$s for caffeine at the three caffeine dose levels were not significantly different by a repeated-measures, one-way ANOVA. $C_{max}$s, the $AUC_{(0-\infty)}$s and $AUC_{(0-24\ h)}$s for caffeine were dose proportional for the three dose levels used. Unlike caffeine, $C_{max}$s and the $AUC_{(0-24\ h)}$s for the three metabolites of caffeine were not dose proportional with respect to caffeine doses. Using the present sampling schedules, the estimated $K_{el}$s for theobromine and paraxanthine decreased with dose, whereas for theophylline it remained the same across the three doses. $AV_d$ and $Cl_{IP}$ values of caffeine were similar across the doses.

*Chronic IP Caffeine*

Table 2 shows the kinetic parameters for caffeine and its three DMXs during 21 days of 20 mg/kg IP administration. The $K_{el}$s of caffeine for the 11th and 21st days were larger than for the acute dose [$F(2, 6) = 5.82$; $p < 0.05$]. Both paraxanthine and theophylline also showed higher values of $K_{el}$ after the 11th injection, whereas theobromine remained the same during this chronic treatment. The $AV_d$ and $Cl_{IP}$ values of caffeine remained similar during this chronic dosing regimen.

*Chronic, Schedule-Induced Oral Self-Administration of Caffeine*

The mean hourly caffeine doses during the 3-h drinking sessions are shown in Fig. 2. The orally self-administered caf-

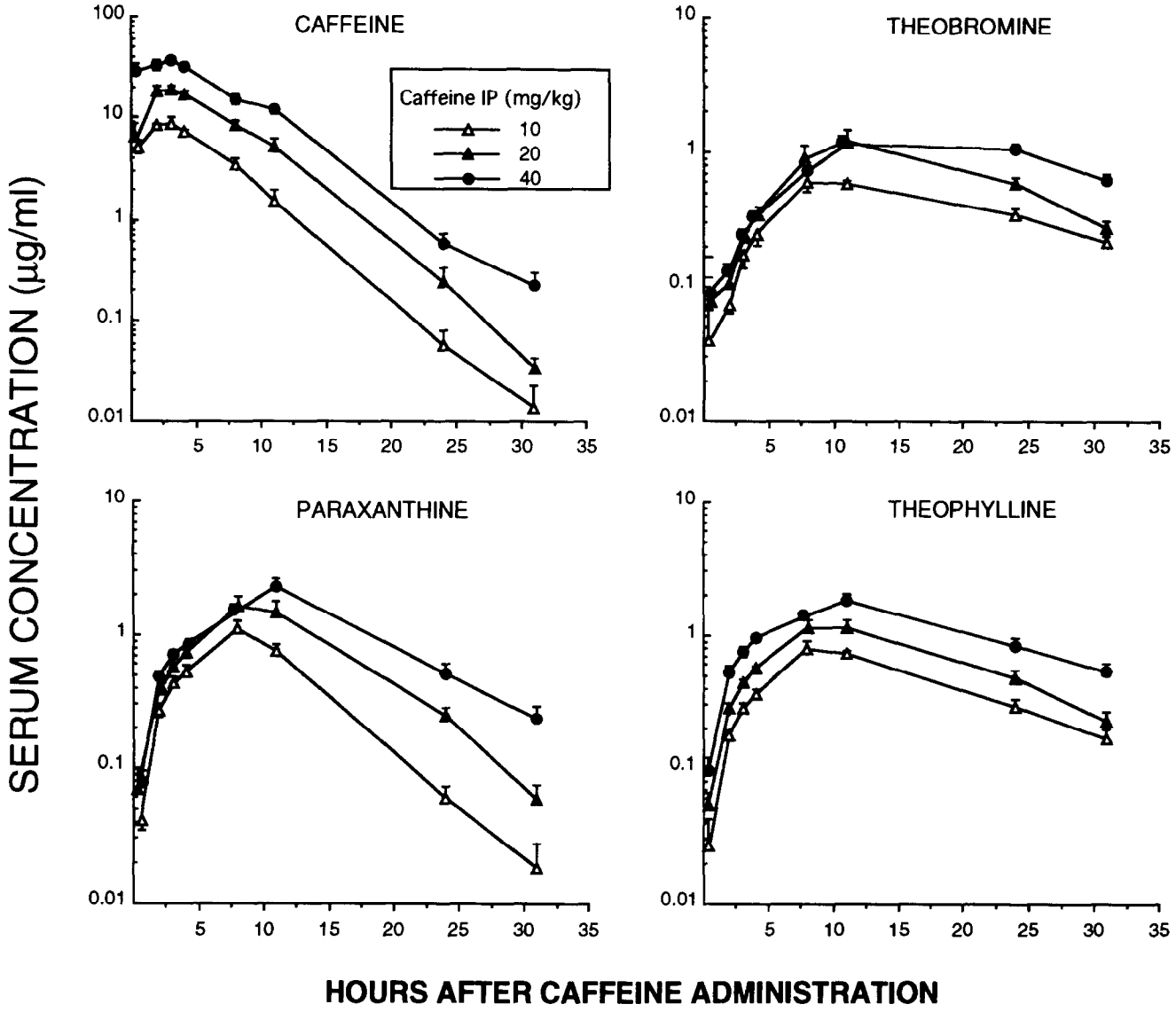


FIG. 1. Mean (SE) concentration-time profiles for serum caffeine and the three dimethylxanthine metabolites after caffeine (10–40 mg/kg, IP) administration. Samples were taken serially from tail tip.

TABLE 1

PHARMACOKINETIC PARAMETERS FOR ACUTE IP CAFFEINE AT THREE DOSE LEVELS IN RATS ($N$ = 4)

| | Dose (mg/kg) | $C_{max}$ (μg/ml) | $K_{el}$/h | $AUC_{(0\text{-}24\ h)}$ (μg × h/ml) | $AUC_{(0\text{-}\infty)}$ (μg × h/ml) | $AV_d$ (liters/kg) | $Cl_{IP}$ (liters/h/kg) |
|---|---|---|---|---|---|---|---|
| Caffeine | 10 | 8.57 | 0.25 | 68.10 | 68.40 | 0.61 | 0.15 |
| | | (±1.35) | (±0.04) | (±7.91) | (±8.03) | (±0.03) | (±0.02) |
| | 20 | 18.81 | 0.23 | 163.55 | 169.18 | 0.53 | 0.12 |
| | | (±1.63) | (±0.01) | (±19.13) | (±20.53) | (±0.06) | (±0.01) |
| | 40 | 37.28 | 0.19 | 341.82 | 345.82 | 0.59 | 0.12 |
| | | (±0.80) | (±0.02) | (±20.90) | (±21.85) | (±0.02) | (±0.01) |
| Theobromine | 10 | 0.61 | 0.05 | 10.10 | | | |
| | | (±0.10) | (±0.01) | (±0.50) | | | |
| | 20 | 1.21 | 0.07 | 18.02 | | | |
| | | (±0.31) | (±0.02) | (±3.44) | | | |
| | 40 | 1.14 | 0.03 | 19.95 | | | |
| | | (±0.13) | (±0.01) | (±1.43) | | | |
| Paraxanthine | 10 | 1.13 | 0.19 | 12.57 | | | |
| | | (±0.17) | (±0.02) | (±0.91) | | | |
| | 20 | 1.66 | 0.15 | 22.25 | | | |
| | | (±0.28) | (±0.01) | (±3.47) | | | |
| | 40 | 2.31 | 0.12 | 30.65 | | | |
| | | (±0.30) | (±0.01) | (±3.04) | | | |
| Theophylline | 10 | 0.81 | 0.07 | 12.12 | | | |
| | | (±0.10) | (±0.01) | (±0.59) | | | |
| | 20 | 1.16 | 0.07 | 18.72 | | | |
| | | (±0.16) | (±0.01) | (±2.10) | | | |
| | 40 | 1.84 | 0.06 | 29.27 | | | |
| | | (±0.22) | (±0.01) | (±2.36) | | | |

feine doses were a linear function of time. The highest self-administered dose was effected by altering the vehicle, rather than by attempting to increase the concentration of the solution. Rats ingested twice the volume when compound solution was used as the vehicle. Serum caffeine concentration-time profiles and the kinetic parameters of caffeine and its three metabolites are shown in Fig. 3 and Table 3. Caffeine concentrations showed dose-related increases from the 1st h and reached $C_{max}$s at the end of the drinking session for the three orally self-administered caffeine doses. It is evident that the food pellets did not interfere with caffeine's absorption and distribution. Formation of the three metabolites was apparent at the 1st h point of the drinking session and continued to form after drinking sessions. The $K_{el}$ of caffeine for the 8.8-mg/kg dose was smaller than for the two higher doses [$F(2, 6) = 10.52$; $p < 0.05$], whereas the $K_{el}$ for 19.4 and 37.9-mg/kg doses were not different from each other. Caffeine and the three metabolites were dose proportional at the three dose levels, as indicated by their $C_{max}$s and $AUC_{(0\text{-}\infty)}$s or $AUC_{(0\text{-}24\ h)}$s. $AV_d$ and $Cl_{po}$ values of caffeine were similar across the oral doses. However, $AV_d$ and Cl values for oral caffeine were two to three times higher than for the IP route [$F(1, 6) = 13.56$; $p < 0.05$; $F(1, 6) = 12.68$; $p < 0.05$, respectively].

## DISCUSSION

In the present study, animals were maintained at 80% of their adult free-feeding body weights. This was done because schedule-induced drinking requires this deprivation condition, but also to permit comparison with the literature on the behavioral effects of caffeine, a literature that often uses behavior motivated by food deprivation as a baseline against which to ascertain the effects of caffeine. For this reason, the pharmacokinetic functions were obtained under the same food-limitation condition. Caffeine is metabolized by hepatic microsomal enzyme systems—that is, the cytochrome P-450 monooxygenase system (1,30) with no significant contribution by other organs (18). Factors affecting these enzyme systems will affect the pharmacokinetics of caffeine. Little research could be located that explored the effect of chronic food-restriction conditions on drug metabolism. A chronic 45% food-restriction condition, compared with the ad lib condition, enhanced drug metabolism by increasing hepatic microsomal drug metabolizing enzymes, and produced a significant decrease in hexobarbital sleeping time in male rats (35). Pretreatment of rats with 3-methylcholanthrene, an inducer of monooxygenase, reduced the AUC of caffeine by 96 and 81% after oral and IV caffeine administration, respectively (1). The elimination of caffeine also changed from nonlinear to an apparently first-order kinetics with $K_{el}$ increased fourfold relative to the value obtained from vehicle animals. Caffeine also can induce the P-450 system and thus increase its own metabolism (30). The $K_{el}$ values of caffeine significantly increased during chronic IP and oral self-administration (Tables 2 and 3). Furthermore, for the dose range of 10–40 mg/kg used in the present study, the $C_{max}$s and the AUCs of caffeine were dose proportional by both routes of administration and showed proportional relations. Thus, the linear kinetics of caffeine also may have resulted from increased drug-metabolizing enzymes in our food-limited rats. Saturation kinetics have been found in rats not deprived of food at doses < 5 mg/kg and plasma concentrations of caffeine as low as 2–4 μg/ml (1,9,25).

Unlike caffeine, the $C_{max}$s and the $AUC_{(0\text{-}24\ h)}$s for the three

TABLE 2

PHARMACOKINETIC PARAMETERS FOR CHRONIC IP 20 mg/kg CAFFEINE AT DIFFERENT DAYS IN RATS ($n$ = 4)

| | $C_{max}$ ($\mu$g/ml) | $K_{el}$/h | $AUC_{(0-24\ h)}$ ($\mu$g × h/ml) | $AUC_{(0-\infty)}$ ($\mu$g × h/ml) | $AV_d$ (liters/kg) | $Cl_{IP}$ (liters/h/kg) |
|---|---|---|---|---|---|---|
| Caffeine | | | | | | |
| Acute | 18.81 | 0.23*† | 163.55 | 169.18 | 0.53 | 0.12 |
| | (±1.63) | (±0.01) | (±19.13) | (±20.53) | (±0.06) | (±0.01) |
| 11th day | 23.29 | 0.27 | 181.35 | 181.66 | 0.43 | 0.11 |
| | (±1.42) | (±0.01) | (±17.80) | (±17.85) | (±0.03) | (±0.01) |
| 21st day | 23.29 | 0.26 | 170.77 | 171.78 | 0.55 | 0.12 |
| | (±2.71) | (±0.01) | (±26.53) | (±30.1) | (±0.09) | (±0.02) |
| Theobromine | | | | | | |
| Acute | 1.21 | 0.07 | 18.02 | 25.77 | | |
| | (±0.31) | (±0.01) | (±3.44) | (±2.73) | | |
| 11th day | 1.83 | 0.07 | 29.77 | 40.98 | | |
| | (±0.08) | (±0.01) | (±0.26) | (±4.21) | | |
| 21st day | 1.47 | 0.06 | 25.26 | 36.98 | | |
| | (±0.30) | (±0.01) | (±4.54) | (±6.91) | | |
| Paraxanthine | | | | | | |
| Acute | 1.66 | 0.15 | 22.25 | 23.75 | | |
| | (±0.28) | (±0.01) | (±3.47) | (±3.61) | | |
| 11th day | 1.97 | 0.19 | 24.47 | 24.99 | | |
| | (±0.05) | (±0.01) | (±1.19) | (±1.19) | | |
| 21st day | 1.60 | 0.14 | 22.58 | 24.37 | | |
| | (±0.25) | (±0.02) | (±3.44) | (±3.69) | | |
| Theophylline | | | | | | |
| Acute | 1.16 | 0.07 | 18.72 | 24.55 | | |
| | (±0.16) | (±0.01) | (±2.10) | (±2.79) | | |
| 11th day | 2.01 | 0.10 | 29.87 | 34.49 | | |
| | (±0.04) | (±0.01) | (±1.33) | (±1.78) | | |
| 21st day | 1.71 | 0.09 | 27.14 | 33.47 | | |
| | (±0.21) | (±0.01) | (±3.52) | (±5.04) | | |

*Differs from 11th day ($p < 0.05$).
†Differs from 21st day ($p < 0.05$).

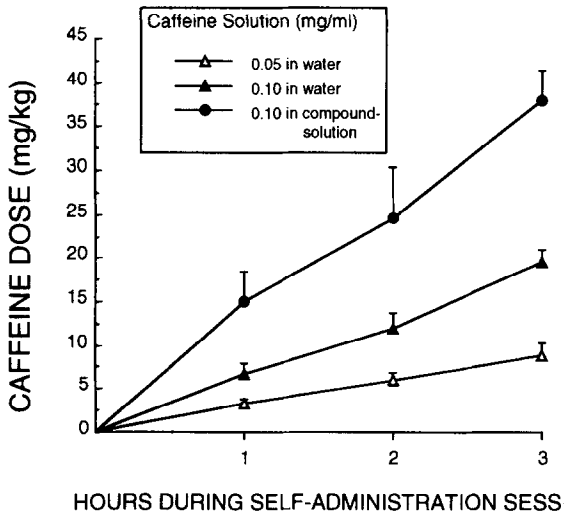


FIG. 2. Mean orally self-administered caffeine doses (SE) during 3-h schedule-induced drinking sessions.

metabolites showed disproportional relations with respect to caffeine doses by the IP route, whereas these metabolites exhibited dose-proportional relations with oral self-administration (Tables 1 and 3). In general, paraxanthine had the largest $K_{el}$ value of the three DMXs, whereas theophylline and theobromine showed the medium and smallest values, respectively, for this parameter for both routes of administration. These three DMXs are further metabolized primarily by demethylation and oxidation by the liver (2,39). The *N*-demethylation pathway responsible for these metabolites is apparently still saturable by the IP route in food-limited rats. These methylxanthines may compete with each other for oxidizing enzyme sites. The linear relations of the three metabolites found in oral self-administration may result from either the slower rate of dose onset by 3-h ingestion than by bolus IP injection, or the increased $Cl_{PO}$ value associated with the oral route.

Caffeine, theophylline, and theobromine are all effective as diuretic agents. They can increase renal blood flow and glomerular filtration rate (34). Although caffeine is a highly metabolized drug with only about 0.5–3% excreted unchanged in urine under normal circumstances (4,7,33,39), high correlations have been found between caffeine renal clearance ($Cl_R$)

and urine flow rate (4,6). Hence, under the schedule-induced oral self-administration condition, the increased urine flow rate may produce an increased caffeine $Cl_R$, which would result in a lower $AUC_{(0-\infty)}$. The smaller values of $AUC_{(0-\infty)}$ for caffeine with oral self-administration may result from the larger values of $Cl_R$. One animal in the oral group had much larger values of $AV_d$ for the two lower caffeine doses, which contributed to the larger standard errors for the group means. However, $K_{el}$ values of caffeine for this animal were not different from the other three animals. As caffeine plasma protein binding is very low, its change does not affect $AV_d$ (9). The increased $AV_d$ values by the oral route may result from an increase in total body water after schedule-induced oral self-administration.

We used a sensitive high performance liquid chromatography method to trace temporal concentration changes of caffeine and its metabolites within the same animals. Using a small species of animal, it is necessary to use a small serum sample size to maximize the number of samples permissible across the 24-h sampling time. If the $K_{el}$ values were similar for caffeine and its metabolites, then all $K_{el}$s could be calculated accurately. However, as caffeine approaches the terminal phase of its elimination, the DMXs are still being formed. It is difficult, with a limited number of permissible samples, to characterize satisfactorily the $K_{el}$s for both caffeine and DMXs. Our main focus was to compare the pharmacokinetics of caffeine in chronic food-limited animals in the present study with information on the kinetics of ad lib animals as reported in the literature. Therefore, we chose our sampling times primarily to evaluate caffeine $K_{el}$s. Nevertheless, $AUC_{(0-24\ h)}$ values allowed us to compare the differences between caffeine and its metabolites with two routes of administration.

The results suggest, in general, that with increasing doses

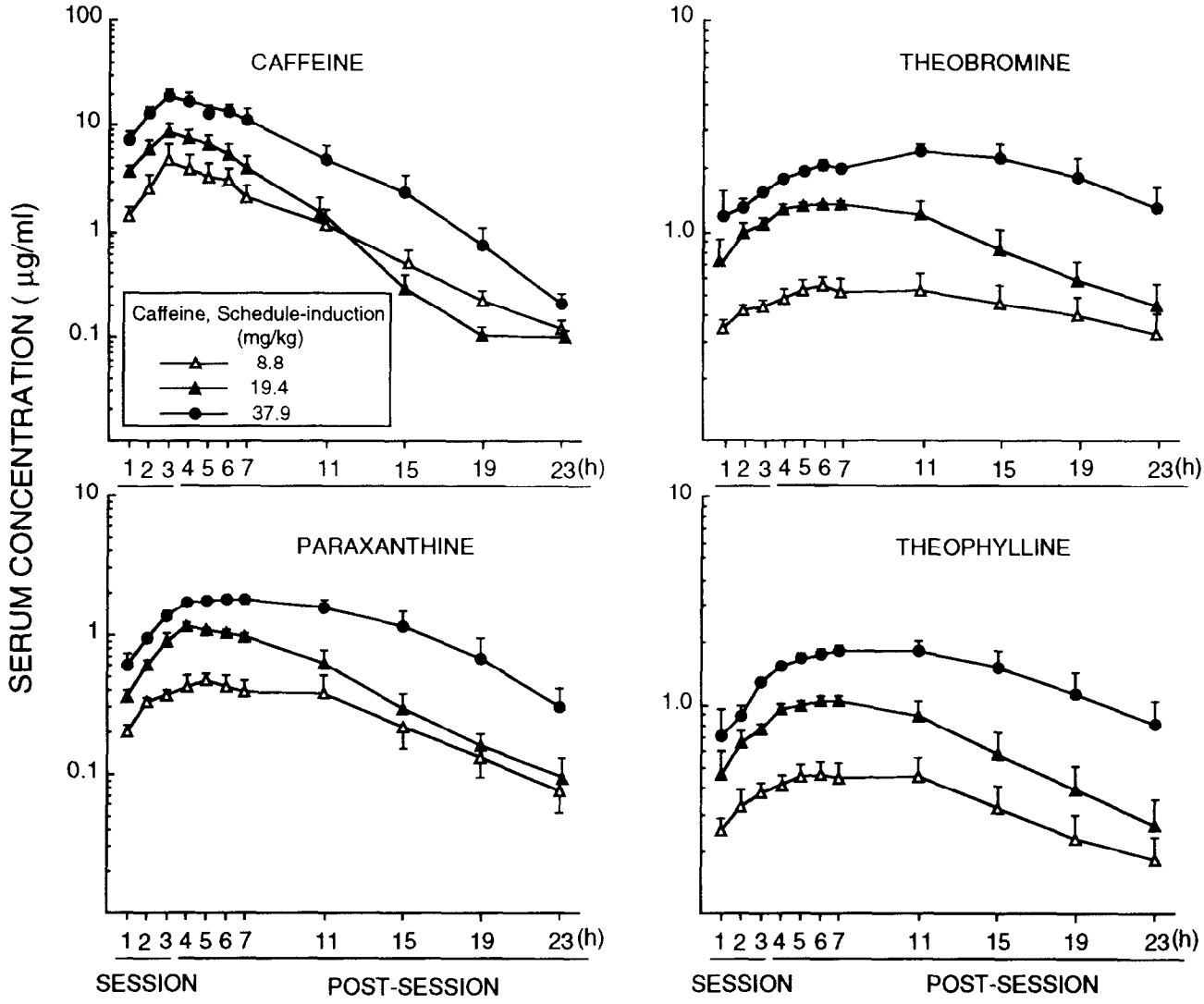


FIG. 3. Mean (SE) concentration-time profiles for serum caffeine and the three metabolites during and after 3-h schedule-induced oral caffeine self-administration sessions at three dose levels. Samples were taken serially from tail tip.

TABLE 3

PHARMACOKINETIC PARAMETERS FOR SCHEDULE-INDUCED ORAL CAFFEINE SELF-ADMINISTRATION AT THREE DOSE LEVELS IN RATS ($n$ = 4)

| | Dose (mg/kg) | $C_{max}$ (μg/ml) | $K_{el}$/h | $AUC_{(0-24\,h)}$ (μg × h/ml) | $AUC_{(0-\infty)}$ (μg × h/ml) | $AV_d$ (liters/kg) | $Cl_{po}$ (liters/h/kg) |
|---|---|---|---|---|---|---|---|
| Caffeine | 8.8 | 4.47 | 0.18*† | 27.57 | 28.69 | 1.85 | 0.33 |
| | (±1.6) | (±1.23) | (±0.01) | (±8.51) | (±7.77) | (±0.40) | (±0.06) |
| | 19.4 | 8.83 | 0.26 | 58.31 | 61.01 | 1.40 | 0.36 |
| | (±1.4) | (±1.64) | (±0.01) | (±12.87) | (±12.69) | (±0.35) | (±0.08) |
| | 37.9 | 18.97 | 0.23 | 136.79 | 138.27 | 1.27 | 0.30 |
| | (±3.6) | (±2.70) | (±0.02) | (±32.28) | (±31.72) | (±0.12) | (±0.05) |
| Theobromine | 8.8 | 0.55 | 0.04 | 9.79 | | | |
| | (±1.6) | (±0.06) | (±0.01) | (±1.73) | | | |
| | 19.4 | 1.36 | 0.09 | 21.92 | | | |
| | (±1.4) | (±0.05) | (±0.02) | (±2.17) | | | |
| | 37.9 | 2.41 | 0.06 | 42.92 | | | |
| | (±3.6) | (±0.17) | (±0.03) | (±4.64) | | | |
| Paraxanthine | 8.8 | 0.47 | 0.12 | 5.85 | | | |
| | (±1.6) | (±0.07) | (±0.02) | (±1.28) | | | |
| | 19.4 | 1.15 | 0.15 | 14.79 | | | |
| | (±1.4) | (±0.07) | (±0.02) | (±0.46) | | | |
| | 37.9 | 1.80 | 0.14 | 25.95 | | | |
| | (±3.6) | (±0.07) | (±0.03) | (±3.24) | | | |
| Theophylline | 8.8 | 0.47 | 0.08 | 7.40 | | | |
| | (±1.6) | (±0.07) | (±0.02) | (±1.53) | | | |
| | 19.4 | 1.04 | 0.09 | 16.14 | | | |
| | (±1.4) | (±0.06) | (±0.03) | (±1.63) | | | |
| | 37.9 | 1.83 | 0.08 | 31.22 | | | |
| | (±3.6) | (±0.22) | (±0.03) | (±4.31) | | | |

*Differs from 19.4 mg/kg ($p < 0.05$).
†Differs from 37.9 mg/kg ($p < 0.05$).

of caffeine, the $K_{el}$ and Cl values remained similar for both routes of administration. These kinetic parameters were in the range found in rats when single doses were used (25,31,40). In the present study, caffeine kinetics exhibited proportional relations in terms of $C_{max}$ and AUC with respect to caffeine doses. During chronic treatment, $K_{el}$s increased for both routes. The linear caffeine kinetics found in chronic food-limited rats contrasts with the nonlinear pharmacokinetics of ad lib rats. The difference between linear and nonlinear kinetics may have behavioral and clinical implications. In humans, high levels of caffeine consumption can lead to an accumulation of serum caffeine and its metabolites, the effects of which are not fully compensated by the development of tolerance (12). It was suggested that this accumulation might explain the nonlinear relation between caffeine dose and response with respect to adverse health effects, such as coronary artery disease (12). Caffeine doses produced a bitonic function for locomotor activity in rodents (20,29,40). Tolerance to the stimulating effect of caffeine on locomotor activity has been shown in rats (10,21,29). Blockade of adenosine receptors by caffeine results in upregulation of the receptors during chronic treatment with caffeine, but this alone cannot fully explain the tolerance (22).

A pharmacologic effect may correlate better with the summed concentrations of the parent compound and its active metabolites than with parent drug concentration alone (24). Because the three DMX metabolites are all pharmacologically active, locomotor activity tolerance to caffeine may be a total methylxanthine dose-response phenomenon rather than a function of caffeine alone (29). Pharmacokinetically, caffeine and the DMXs will remain longer and lead to a higher concentration if caffeine kinetics are nonlinear. A relative depressant effect may result from increased concentrations of methylxanthines, and that may partially account for the decrease in activity that is interpreted as tolerance. This notion was supported by the finding that tolerance to the increased rate of locomotor activity produced by caffeine occurred in fewer days with daily IP 20 mg/kg caffeine doses than with chronic, daily oral caffeine self-administration, 36.5 mg/kg, in food-limited rats, whereas at lower oral doses no tolerance was observed (29).

Tolerance was demonstrated even after 1 day of exposure to oral caffeine in ad lib rats (16), whereas tolerance occurred only after 13 days by oral-self administration at 36.5 mg/kg (range 34.3-39.2) in food-limited rats (29). Thus, the pharmacodynamics of caffeine may differ significantly between food-limited and ad lib animals because of differences in the pharmacokinetics between these two feeding regimens.

ACKNOWLEDGEMENTS

This work was supported by Grants R01 DA 05305 and K05 DA00142 from the National Institute on Drug Abuse.

## REFERENCES


1. Aldridge, A.; Parsons, W. D.; Neims, A. H. Stimulation of caffeine metabolism in the rat by 3-methylcholanthrene. Life Sci. 21: 967-974; 1977.
2. Arnaud, M. J. Metabolism of caffeine and other components of coffee. In: Garattin, S., ed. Caffeine, coffee, and health. New York: Raven Press; 1993:43-95.
3. Axelrod, J.; Reichental, J. The fate of caffeine in man and a method for its estimation in biological material. J. Pharmacol. Exp. Ther. 107:519-523; 1953.
4. Birkett, D. J.; Miners, J. O. Caffeine renal clearance and urine caffeine concentrations during steady state dosing. Implications for monitoring caffeine intake during sports events. Br. J. Clin. Pharmac. 31:405-408; 1981.
5. Blanchard, J.; Sawers, S. J. A. The absolute bioavailability of caffeine in man. Eur. J. Clin. Pharmacol. 24:93-98; 1983a.
6. Blanchard, J.; Sawers, S. J. A. Relationship between urine flow rate and renal clearance of caffeine in man. J. Clin. Pharmacol. 23:134-138; 1983b.
7. Bonati, M.; Latini, R.; Galletti, F.; Young, J. F.; Tognoni, G.; Garattini, S. Caffeine disposition after oral doses. Clin. Pharmcol. Ther. 32:98-106; 1982.
8. Bonati, M.; Garattini, S. Interspecies comparison of caffeine disposition. In: Dews, P. B., ed. Caffeine. New York: Springer-Verlag; 1984:48-56.
9. Burg, A. W. Physiological disposition of caffeine. Drug Metab. Rev. 4:199-228; 1975.
10. Chou, D. T.; Khan, J. F.; Hirsh, K. R. Caffeine tolerance: Behavioral, electrophysiological and neurochemical evidence. Life Sci. 36:2347-2358; 1985.
11. Denaro, C. P.; Brown C. R.; Wilson, M. S.; Jacob, P. III; Benowitz, N. L. Dose-dependency of caffeine metabolism with repeated dosing. Clin. Pharmacol. Ther. 48:277-285; 1990.
12. Denaro, C. P.; Brown C. R.; Wilson, M. S; Jacob, P. III; Benowitz, N. L. Effects of caffeine with repeated dosing. Eur. J. Clin. Pharmacol. 40:273-278; 1991.
13. Falk, J. L. Production of polydipsia in normal rats by an intermittent food schedule. Science (Wash. D.C.) 133:195-196; 1961.
14. Falk J. L.; Tang, M. Schedule induction of drug intake: Differential responsiveness to agents with abuse potential. J. Pharmacol. Exp. Ther. 249:143-148; 1989.
15. Falk, J. L.; Ma, F.; Lau, C. E. Chronic oral cocaine self-administration: Pharmacokinetics and effects on spontaneous and discriminative motor functions. J. Pharmacol. Exp. Ther. 257:457-465; 1991.
16. Finn, I. B.; Holtzman, S. G. Tolerance to caffeine-induced stimulation of locomotor activity in rats. J. Pharmacol. Exp. Ther. 238:542-546;1986.
17. Gilbert, R. M. Caffeine as a drug of abuse. In: Gibbons, R. J.; Israel Y.; Kalant, M.; Poplan, R. E.; Schmidt, W.; Smart, R. G., eds. Research advances in alcohol and drug problems, vol. 3. New York: John Wiley & Sons; 1976:49-176.
18. Grant, D. M.; Campbell, M. E.; Tang, B. K.; Kalow, W. Biotransformation of caffeine by microsomes from human liver. Kinetics and inhibition studies. Biochem. Pharm. 36:1251-1260; 1987.
19. Hirsh, K. Central nervous system pharmacology of the dietary methylxanthines. In: Spiller, G. S., ed. Progress in clinical and biological research. vol. 158. New York: Alan R. Liss, Inc.; 1984: 235-302.
20. Hirsh , K. R.; Pinzone, M. G.; Forde, J. H. Spontaneous locomotor activity changes evoked by caffeine. Fed. Proc. 33:466; 1974.
21. Holtzman, S. G. Complete, reversible drug-specific tolerance to stimulation of locomotor activity by caffeine. Life Sci. 33:779-787; 1983.
22. Holtzman, S. G.; Mante, S.; Minneman, K. P. Role of adenosine receptors in caffeine tolerance. J. Pharmacol. Exp. Ther. 256:62-68; 1991.
23. Kaplan, G. B.; Greenblatt, D.; Kent, M. A.; Cotreau-Bibbo, M. M. Caffeine treatment and withdrawal in mice: Relationships between dosage, concentrations, locomotor activity and A1 adenosine receptor binding. Pharmacol. Exp. Ther. 266:1563-1572; 1993.
24. Klockowski, P. M.; Levy, G. Kinetics of drug action in disease states. XXIV. Pharmacodynamics of diazepam and its metabolites in rats. J. Pharmacol. Exp. Ther. 244:912-918; 1988.
25. Latini, R.; Bonati, M.; Castelli, D.; Garattini, S. Dose-dependent kinetics of caffeine in rats. Toxicol. Lett. 2:267-270; 1978.
26. Lau, C. E.; Falk, J. L. Sustained synergism by chronic caffeine of the motor control deficit produced by midazolam. Pharmacol. Biochem. Behav. 40:723-731;1991.
27. Lau, C. E.; Falk, J. L.; Dolan, S.; Tang, M. Simultaneous determination of flurazepam and five metabolites in serum by high-performance liquid chromatography and its application to pharmacokinetic studies in rats. J. Chromatogr. 423:251-259; 1987.
28. Lau, C. E.; Imam; A.; Ma, F.; Falk, J. L. Acute effects of cocaine on spontaneous and discriminative motor functions: Relation to route of administration and pharmacokinetics. J. Pharmacol. Exp. Ther. 257:444-456; 1991.
29. Lau, C. E.; Falk, J. L. Tolerance to oral and IP caffeine: Locomotor activity and pharmacokinetics. Pharmacol. Biochem. Behav. 48:337-344; 1994.
30. Mitoma, C.; Lombrozo, L.; Le Valley, S. E.; Dehn, F. Nature of the effect of caffeine on the drug-metabolizing enzymes. Arch. Biochem. Biophys. 134:434-441; 1969.
31. Nakazawa, K.; Tanaka, H.; Arima, M. The effect of caffeine ingestion on pharmacokinetics of caffeine and its metabolites after a single administration in pregnant rats. J. Pharmacobiodyn. 8:151-160; 1985.
32. Neims, A. H.; von Borstel, R. W. Metabolism and biochemical mechanisms of action. In: Wurtman, R. J.; Wurtman, J. J., eds. Nutrition and the brain, vol. 6. New York: Raven Press; 1983:1-30.
33. Newton, R.; Broughton, L. J.; Lind, M. J.; Morrison, P. J.; Rogers, H. J.; Bradbrook, I. D. Plasma and salivary pharmacokinetics of caffeine in man. Eur. J. Clin. Pharmacol. 21:45-52; 1981.
34. Rall, W. Central nervous system stimulants: The methylxanthines. In: Gillman, A. G.; Goodman, J. S.; Rall, T. W.; Murad, F., eds. The pharmacological basis of therapeutics. New York: MacMillan; 1985:589-603.
35. Sachan, D. S. Modulation of drug metabolism by food restriction in male rats. Biochem. Biophys. Res. Commun. 104:984-989; 1982.
36. Spiller, G. A. Overview of the methylxanthine beverages and foods and their effect on health. In: Spiller, G. A., ed. Progress in clinical and biological research, vol. 158. New York: Alan R. Liss, Inc.; 1984:1-7.
37. Starvic, B. Methylxanthines: Toxicity to humans. I. Theophylline. Food Chem. Toxicol. 26:541-565; 1988a.
38. Starvic, B. Methylxanthines: Toxicity to humans. III. Theobromine, paraxanthine and the combined effects of methylxanthines. Food Chem. Toxicol. 26:725-733; 1988b.
39. Tang-Liu, D. D.; Williams, R. L.; Riegelman, S. Disposition of caffeine and its metabolites in man. J. Pharmacol. Exp. Ther. 224:180-185; 1983.
40. Thithapandha, A.; Maling, H. M.; Gillette, J. R. Effects of caffeine and theophylline on activity of rats in relation to brain xanthine concentrations. Proc. Soc. Exp. Biol. Med. 139:582-586; 1972.