Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 2, pp. 337-344, 1994

0091-3057/94 $6.00 + .00

0091-3057(93)E0032-Y

Tolerance to Oral and IP Caffeine: Locomotor Activity and Pharmacokinetics

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Received 21 October 1993

LAU, C. E. AND J. L. FALK. *Tolerance to oral and IP caffeine: Locomotor activity and pharmacokinetics.* PHARMACOL BIOCHEM BEHAV **48**(2) 337-344, 1994.—Locomotor activity increase was a bitonic function of acute caffeine IP doses (2.5-40 mg/kg) in rats. When the schedule-induced polydipsic, orally self-administered dose of caffeine was increased over blocks of daily 3 h sessions from 9.3 to 36.5 mg/kg, postsession activity increased monotonically as a function of dose. The rate of tolerance development to the increase in locomotor activity produced by caffeine depended on the route of administration. Tolerance onset occurred on the fourth day of chronic IP doses, but remained incomplete after 21 doses. With the highest dose level of oral caffeine self-administration, tolerance developed on day 13, but remained incomplete even after 17 doses. Acute tolerance occurred for each of the IP doses, whereas a linear relation between locomotor activity and serum caffeine concentration was obtained after oral self-administration. There were two- to threefold higher locomotor activity $AUCs_{(4\,h)}$ with oral caffeine at three dose levels compared to the activity $AUCs_{(4\,h)}$ for IP doses.

Caffeine tolerance Locomotor activity Caffeine pharmacokinetics Dimethylxanthines

CAFFEINE (1,3,7-trimethylxanthine) is the psychoactive agent most widely self-administered by humans (14). It has pharmacological effects as a central nervous stimulant, cardiotonic, and diuretic (26,28). Caffeine produced dose-related increases in locomotor activity in rodents (3,18,32). Tolerance to the behavioral effects of caffeine has been described for rats (6,15,16,23), mice (27), and humans (9,29). Mechanisms underlying tolerance to the increased locomotor activity produced by caffeine following chronic administration are not well understood. Caffeine and other methylxanthines have antagonist actions at brain adenosine receptor sites (30,31). The upregulation of adenosine receptors with repeated caffeine dosing (2,25) may be important as a basis for caffeine tolerance, but this alone does not fully explain the tolerance observed (13,17).

It is often assumed that drug action is linearly related to drug level in the central systemic circulating compartment. Frequently, it is not (4). If the peak effect occurs later than the drug concentration peak, the situation is one of hysteresis. If the peak effect occurs before the drug concentration peak, the situation is one of proteresis. In the hysteresis case, a plot of effect vs. drug concentration (connected in time order) shows an anticlockwise loop, referred to as anticlockwise hysteresis; in the case of proteresis, the same plot shows a clockwise loop, referred to as clockwise hysteresis. If equilibrium exists between the drug levels in plasma and at the site of action, then there is more likely to be a direct relation between plasma drug level and its action. However, active metabolites of the parent compound also can alter the pharmacological response following the drug administration. Pharmacological effect may correlate better with the sum concentrations of the parent drug and its active metabolites rather than with parent drug concentration alone. For example, the active metabolite of diazepam, desmethyldiazepam, contributes significantly to the hypnotic activity of diazepam in humans (19). Caffeine is biotransformed to 28 metabolites that have been detected in the urine of animals and humans (1). The three dimethylxanthine metabolites of caffeine, theobromine, paraxanthine, and theophylline, are all pharmacologically active (7). The aim of the present study was to investigate caffeine's effects on locomotor activity in rats by two routes of administration, IP and schedule-induced oral self-administration, and to relate the locomotor effects to the respective pharmacokinetic profiles. In addition, the role of the dimethylxanthines in the possible development of tolerance to the effects of caffeine administration was of interest.

METHOD

Locomotor Activity

Animals. Five adult male, albino rats of the Holtzman strain (Madison, WI) with a mean initial body weight of 386 g

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(range: 381–391 g) were housed individually in stainless steel cages in a temperature-regulated room with a 12 L : 12 D cycle (lights on 0700–1900 h).

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

Apparatus. Spontaneous locomotor activity was measured, as described previously (11,22), in a room isolated from other activities and noise. Animals were placed individually into stainless steel cages (38.0 × 25.5 × 17.5 cm) resting on Startle-Tremor Platforms (E45-10, Coulborn Instruments, Allentown, PA). The platforms were connected to individual activity monitors (E61-11) which were located in an adjacent room. Each monitor was threshold adjusted, by means of its sensitivity and separation controls, to sort a platform movement onto one of two electronic counters. The large-movement counter used in this study recorded larger movements constituting locomotion, but not smaller movements (grooming, sniffing, etc.). The data were collected with an IBM-compatible computer through a Lab Linc interface (Coulborn Instruments) with the activity monitors.

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 (11). 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 (a FT 1 min schedule). Under this sort of food-delivery schedule, polydipsia occurs during each session (10). In the present experiment, the food-delivery schedule induced the oral self-administration of high volumes of caffeine solutions. At the end of each session, fluid intakes were recorded and animals were transferred immediately into a locomotor activity evaluation session.

Procedure. After establishing ad lib weights, animals were reduced to 80% body weight 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, 8 h activity-monitoring sessions were conducted daily (7 days per week) for the entire series of experiments. Immediately before each daily session, animals were weighed, transported to the experimental room, and placed into their individual activity-monitoring cages at 1600 h and remained there overnight. At 0900 h, animals were returned to their home cages and given food rations to maintain their criterion weights. After 8 to 14 days of daily activity measurement, all animals displayed low intersession variability and drug treatments commenced. Injections were given immediately before an animal was placed into its activity-monitoring cage.

First, the effect of acute IP caffeine injections on activity was evaluated. Each animal received three vehicle injections separated by 3 days. They then received doses of 2.5, 5, 10, 20, and 40 mg/kg caffeine in an ascending dose order, with injections separated by 3 to 7 days. Ten days after the last acute caffeine dose, animals received chronic IP injections of 20 mg/kg caffeine for 21 days.

The animals then were exposed to the second chronic caffeine procedure, in which daily 3 h schedule-induced self-administration sessions immediately preceded their transfer at 1600 h every day into an activity evaluation session. The fluid available during the schedule-induction session was distilled water for the first 15 days. Then, the following sequence of session fluids was available: 0.05 mg/ml caffeine (6 days); 0.1 mg/ml caffeine (14 days); 0.1 mg/ml caffeine in a compound-solution vehicle [0.08% sodium saccharin and 1.5% glucose (17 days)]; compound solution (4 days). Under these conditions, three dose levels of oral caffeine were self-administered. At the end of the 8 h activity session, animals remained in the activity cages overnight and were then returned to their home cages and given food rations sufficient to maintain their criterion weights at 0900 h. The 9 h delay was included to ensure that no prefeeding increase in activity would occur toward the end of the 8 h activity measurement period which could have confounded the evaluation of drug-induced changes in activity.

Pharmacokinetics

Animals. Eight male albino rats (Holtzman strain) with a mean initial body weight of 384 g (range: 383–385 g; age: 6–9 months) were used. The living conditions and feeding regimen were those of the previous experiment.

Reagents, serum sampling, and HPLC. Theobromine, paraxanthine, and theophylline were purchased from Sigma Chemical Co. (St. Louis, MO). Other reagents were obtained from standard commercial sources. The serum sampling procedure and a sensitive serum microsample (25 or 50 μl) HPLC method used for determination of caffeine and its metabolites has been described previously (20,21).

Procedure. The first group (n = 4) received four acute IP caffeine doses in an ascending order: 5, 10, 20, and 40 mg/kg. Blood samples were collected at 0.25, 0.5, 0.75, 1, 2, 3, and 4 h. Ten days after the last acute caffeine dose, daily 20 mg/kg caffeine IP doses were administered to animals for 6 days. Blood samples were taken on the first, second, third, and sixth day at the same time intervals as in the acute phase.

The second group of animals (n = 4) was used to study the serum caffeine concentration profiles with the schedule-induced oral self-administration method. Animals were given daily 3 h drinking sessions as described above. 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. Blood samples (100 μl) were drawn immediately after selected sessions, at time 0 (i.e., after 3 h of drinking a caffeine solution), and again at hours 1, 2, 3, and 4 after the end of the self-administration sessions. 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 the sixth day), 0.1 mg/ml caffeine (8 days, sampled on the sixth day), 0.1 mg/ml caffeine in compound-solution vehicle [0.08% sodium saccharin and 1.5% glucose (9 days, sampled on the sixth day)].

Data analysis. The area under the curve (AUC) was calculated by the trapezoidal method. For schedule-induced oral caffeine self-administration, the serum caffeine concentrations were measured at the time points described above. To relate all locomotor activity data points to the respective caffeine serum concentration time data points, the 0.25, 0.5, and 0.75 h serum caffeine concentrations for the three oral dose levels, and the 2 h point for the 37.9 mg/kg caffeine dose, were calculated from the serum concentration-time profiles.

They were calculated from $C_{(t)} = C_0e^{-kelt}$ after 3 h of oral caffeine self-administration, where elimination rate constants (kel) were computed by log-linear regression analysis with assumption of a first-order, one-compartment, open model, and C_0 was the serum concentration at time zero, i.e., immediately after the caffeine-drinking session.

Statistical analyses of the activity data were performed by repeated-measures, two-way ANOVA with treatment and time as factors within subjects. Repeated-measures, one-way ANOVA with treatment as the factor also was used. Paired *t*-tests, correlation coefficients, and regression analyses also were performed as appropriate.

RESULTS

Locomotor Activity

Acute IP caffeine. Figure 1 shows the mean locomotor activity rate-time profiles as a function of caffeine IP doses from 0.25 to 4 h after injection, $F(36, 144) = 1.90$, $p < 0.005$. Baseline activity rate for each time point was calculated as the grand mean of the 2-day nontreatment means that preceded each injection. There are dose-dependent differences in activity rates, $F(6, 24) = 3.58$, $p < 0.05$, with the highest activity rate at 20 mg/kg, which is significantly higher than both the 10 and 40 mg/kg doses. The threshold effective caffeine dose that increased the locomotor-activity rate profile was 10 mg/kg. Activity rate was also time dependent, $F(6, 24) = 13.46$, $p < 0.001$, with the highest activity at 0.25 h and a second peak at 1 h, followed by a slow decrease to baseline level by the third hour.

Chronic IP caffeine. Figure 2 shows the 4 h activity rate for the first 7 days of daily IP 20 mg/kg caffeine administration, as well as days 11 and 21. There was a significant decrease in activity rate, $F(6, 24) = 2.76$, $p < 0.05$, during this chronic injection series. Statistically significant tolerance developed on the fourth day of chronic caffeine administration and activity rate remained low through the twenty-first day. However, tolerance was not complete. Locomotor activity at the day 21 point is significantly higher than the baseline point B ($p < 0.05$).

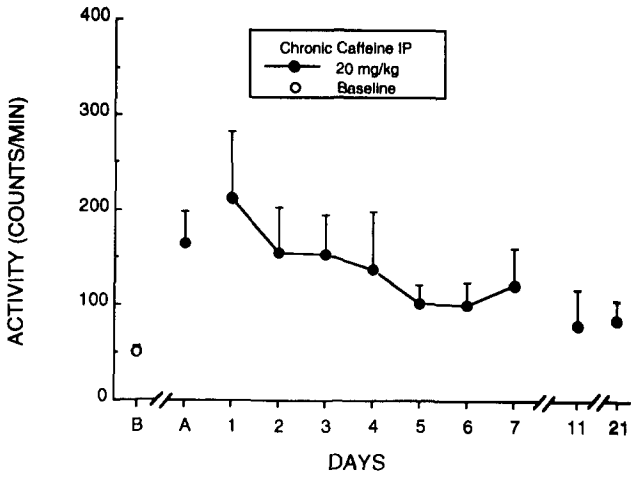

FIG. 2. Mean (SE) effects on 4 h activity rates of chronic 20 mg/kg IP caffeine ($n = 5$). (A) Acute dose of 20 mg/kg.

Chronic, schedule-induced, oral self-administration of caffeine. Following schedule-induced oral caffeine self-administration, locomotor activity increased significantly as a function of dose (Fig. 3). The activity profile produced by the 9.3 mg/kg dose (mean of 6 days) did not differ from the water control profile. However, both the water- and compound-solution profiles were elevated compared to the baseline profile, particularly at the 0.25 h point. The preceding 3 h schedule-induction session itself, then, increased the ensuing level of activity. Nevertheless, for the 9.3 mg/kg dose, the 0.25 h point approached a significant difference when compared to the corresponding value for water ($p < 0.07$). The profiles for the 19.3 mg/kg (mean of 14 days) and 36.5 mg/kg (mean of 6 days) doses were elevated above the other profiles, but did not differ from each other. The baseline is the 3 day mean

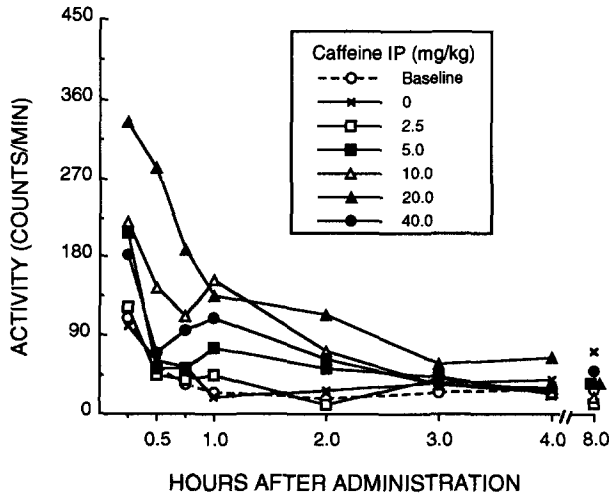

FIG. 1. Mean time profiles of locomotor activity rates for acute IP doses of caffeine ($n = 5$).

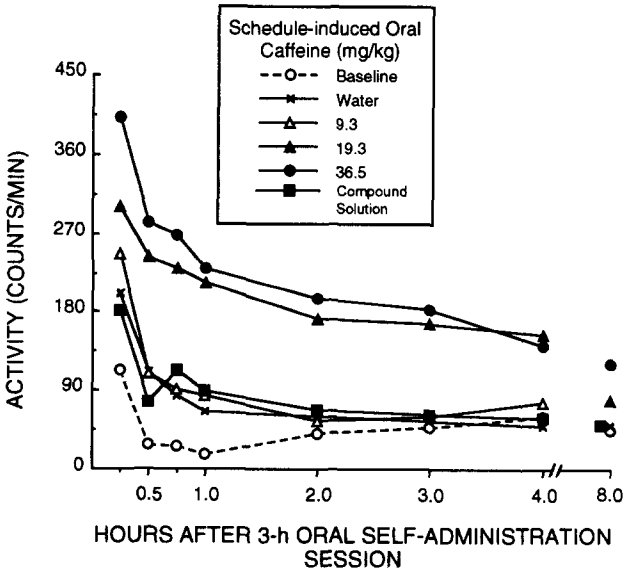

FIG. 3. Mean time profiles of locomotor activity rates for 3 h schedule-induced oral self-administration of caffeine ($n = 5$).

of activity rates prior to the initiation of schedule-induced drinking sessions, whereas the values under water (5 day mean) and compound-solution (4 day mean) conditions were the vehicle controls for before and after the chronic caffeine oral self-administration series, respectively, as described in the Method section. The highest locomotor activity rates were observed at 0.25 h for all the treatments and decreased as a function of time, $F(6, 24) = 12.27$, $p < 0.0001$. There was a further decrease from 4 to 8 h for the median dose, whereas activity for the largest dose remained elevated.

Although the activity rate at the lowest caffeine dose, 9.3 mg/kg, was equivalent to the rate when water had been the session fluid, activity increased when the dose drunk increased to 19.3 mg/kg. This increased activity was sustained over the 14 day period for which this median dose level condition was maintained. The highest caffeine dose, 36.5 mg/kg, did not further increase the activity rate, but after 13 days tolerance developed as shown by regression analysis [$Y = 234.43 - 7.92X$; $r^2 = 0.12$, $p < 0.005$; $F(1, 63) = 8.6$, $p < 0.005$] and Fig. 4. Although locomotor activity rate was significantly lower by day 13, compared to the first day at that dose, tolerance was not complete, even at the end of the caffeine self-administration series on day 17.

Pharmacokinetics

Acute IP caffeine. Serum caffeine concentration-time profiles at four acute caffeine doses (5, 10, 20 and 40 mg/kg) are shown in Fig. 5A. The $AUCs_{(0\text{-}4\ h)}$ for caffeine and its three dimethylxanthines are shown in Table 1. There is a linear relation between caffeine dose and $AUC_{(0\text{-}4\ h)}$ [$r^2 = 0.948$, $F(1, 14) = 254.72$, $p < 0.0001$]. Unlike caffeine, the $AUC_{(0\text{-}4\ h)}$ values for the three metabolites of caffeine were nonlinear with caffeine doses.

Chronic, schedule-induced oral self-administration of caffeine. Serum caffeine concentration-time profiles and the $AUC_{(0\text{-}4\ h)}$ values for caffeine and its three metabolites are shown in Fig. 5B and Table 1. The $AUC_{(0\text{-}4\ h)}$ values of caffeine and the three metabolites all exhibted linear relations as a function of dose.

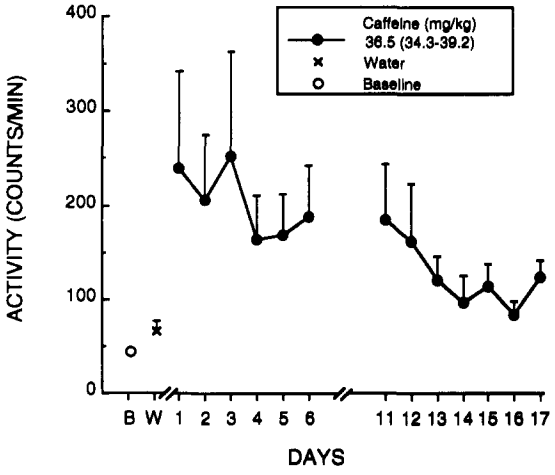

FIG. 4. Mean (SE) effects of daily 3-h schedule-induced oral self-administration of caffeine on 4-h activity rates ($n = 5$).

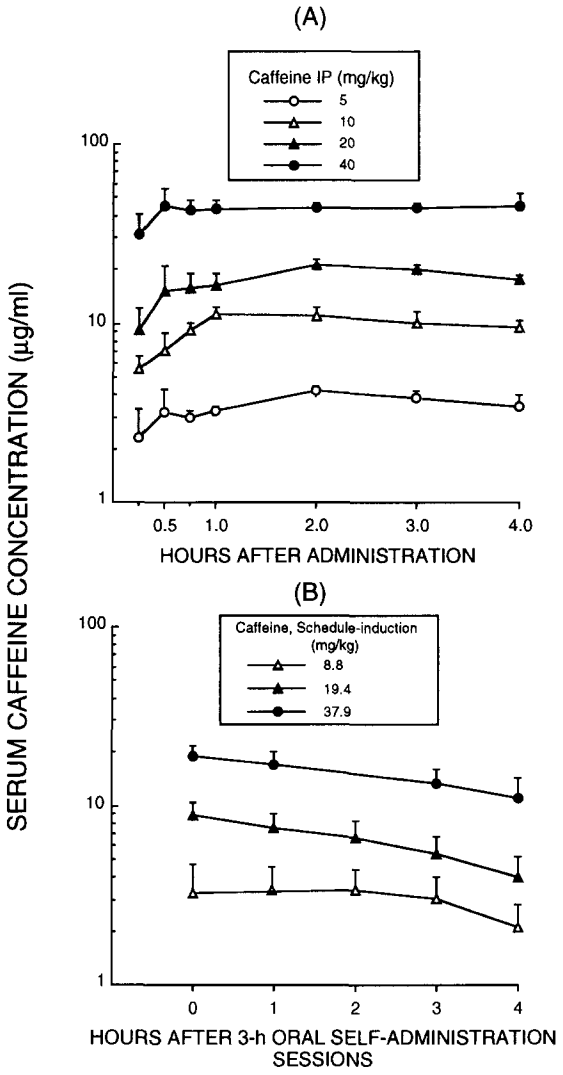

FIG. 5. (A) Mean (SE) concentration-time profiles for serum caffeine after caffeine (5–40 mg/kg) IP administration. (B) Mean (SE) concentration-time profiles for serum caffeine after 3 h schedule-induced oral self-administration of caffeine (8.8–37.9 mg/kg) administration. Samples taken serially from tail tip.

Locomotor Activity and Pharmacokinetics

$AUC_{activity}$ and $AUC_{serum\text{-}concentration}$. Figure 6 shows the 4 h locomotor activities plotted as a function of the serum caffeine AUCs after IP administration and schedule-induction sessions. The 4 h postdose $AUCs_{activity}$ for IP and schedule induction considered across similar ranges of serum caffeine AUCs (14–70 for IP and 9–59 for schedule induction) in the left half of the figure differ, $F(3, 12) = 7.98$, $p < 0.05$. The oral self-administration of caffeine led to considerably greater locomotor activity than did IP imposition at comparable serum caffeine AUCs. For example, although the serum caffeine AUC for schedule-induction (26 µg × h/ml) was somewhat lower than the caffeine AUC for IP (39 µg × h/ml), nevertheless, the activity AUC was almost three times greater ($p < 0.05$).

Table 2 shows that the $AUCs_{activity(0\text{-}4\ h)}$ decreased with daily

TABLE 1

$AUC_{(0\text{-}4\ h)}$ FOR CAFFEINE AND ITS THREE METABOLITES

Dose (mg/kg)	Caffeine	Theobromine	Paraxanthine	Theophylline
		(μg × h/ml)		
		IP		
5	13.94 (±5.31)	1.30 (±0.34)	1.34 (±0.19)	0.79 (±0.23)
10	38.62 (±3.07)	2.03 (±0.66)	2.22 (±0.68)	1.04 (±0.30)
20	70.01 (±2.65)	2.17 (±0.61)	2.79 (±0.72)	1.52 (±0.17)
40	167.31 (±16.55)	2.57 (±0.80)	3.51 (±0.96)	2.52 (±0.69)
		Schedule-Induced Drinking		
8.8 (±1.6)	13.59 (±4.05)	2.05 (±0.22)	1.70 (±0.24)	1.76 (±0.23)
19.4 (±1.4)	25.91 (±5.78)	5.19 (±0.21)	4.20 (±0.17)	3.89 (±0.21)
37.9 (±3.6)	60.70 (±11.57)	7.61 (±0.20)	6.80 (±0.21)	6.50 (±0.26)

chronic IP 20 mg/kg caffeine administration. The serum $AUC_{(0\text{-}4\ h)}$ values for caffeine and the three metabolites increased by the second or third day and remained stable for the entire series. The serum $AUC_{(0\text{-}4\ h)}$ for the three metabolites increased 1.5 to 2.8 times at the second day, whereas for caffeine only by 1.1 at the third day. The serum $AUC_{(0\text{-}4\ h)}$ values for caffeine and the three metabolites for the first day of chronic caffeine dosing were very close to the values for the acute 20 mg/kg dose shown in Table 1. This suggests that 10 days after completion of the acute caffeine administration series there were no residual serum methylxanthines. Locomotor $AUCs_{activity(0\text{-}4\ h)}$ decreased as the combined $AUCs_{(0\text{-}4\ h)}$ for caffeine and the three active metabolites increased over the days shown in Table 2, $r^2 = 0.586$, $F(1, 4) = 5.67$, $p = 0.076$. The values in Table 2 used for serum $AUCs_{(0\text{-}4\ h)}$ of the methylxanthines for eleventh and twenty-first day came from different groups of animals ($n = 4$) with same treatments as the first group described in the Method section except serum samples were obtained at different days (unpublished data).

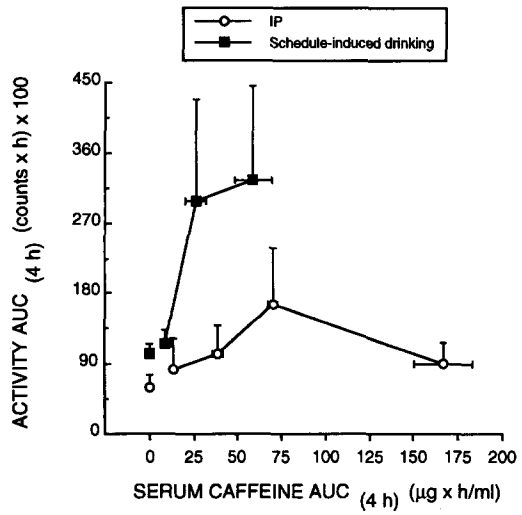

FIG. 6. Mean (SE) locomotor activity $AUC_{(0\text{-}4\ h)}$ values plotted as a function of mean serum caffeine $AUC_{(0\text{-}4\ h)}$ for the 4 IP doses (5–40 mg/kg) and $AUC_{(0\text{-}4\ h)}$ for the schedule-induced oral caffeine intakes at three dose levels. Activity AUCs calculated from total counts at each time interval.

Relation between locomotor activity rate and serum caffeine concentration. The relation between locomotor activity and serum caffeine concentration depends on the route of administration. Figure 7 shows activity in relation to serum caffeine concentration at sequential times from 0.25 to 4 h after administration for the four acute IP caffeine doses. The clockwise hysteresis effects reveal the acute tolerance to the increased locomotor activity produced by a single dose of caffeine. The effects are apparent for all four doses. When caffeine was orally self-administered, by the end of a 3 h caffeine solution drinking session, sufficient time had elapsed for caffeine to have reached a distribution equilibrium in the body. Under these conditions, locomotor activity was a direct linear function of serum caffeine concentrations ($r^2 = 0.61$, $p < 0.0001$) as shown in Fig. 8. Inasmuch as serum samples were not obtained at 0.25, 0.5, and 0.75 h, serum concentrations for these times were calculated as described in the Method section. The relation predicts that when serum caffeine concentration is zero, the activity should be 85.4. The prediction agrees with the value observed, 84, under the water-polydipsia condition shown in Figure 4.

DISCUSSION

Caffeine was more effective in producing increases in locomotor activity by the schedule-induced oral self-administration procedure than by the IP route of administration (Fig. 6). The activity rate after oral self-administration remained high for 8 h, especially for the highest caffeine dose (36.5 mg/kg, Fig. 3), whereas for the IP route, activity rates decreased to baseline level by 3 h for all caffeine doses (Fig. 1). Caffeine produced a depressant effect at the highest IP dose (40 mg/kg). The depressant effects of high doses of caffeine and other xanthines on behavior have been attributed to their inhibition of cyclic adenosine monophosphate phosphodiesterase (5).

Tolerance to the stimulating effect of caffeine on locomotor activity has been shown in rodents (6,15,16). Blockade of adenosine receptors by caffeine results in upregulation of the receptors during chronic treatment with caffeine, but this alone does not fully explain the tolerance (7). Tolerance could be demonstrated even after 1 day of exposure to caffeine orally and was lost 3 to 4 days after the cessation of drug treatment (12). In the present study, caffeine IP doses were separated by 3 to 10 days to avoid the effect of tolerance on the determination of the acute dose-effect curve. With daily 20 mg/kg IP injections, tolerance developed by the fourth day, but even after 21 days of injection, tolerance was not complete. With oral self-administration at the highest caffeine dose, 36.5 mg/kg, tolerance developed only after about 2 weeks of exposure, although, again, tolerance remained incomplete. For both routes of administration activity rates after tolerance had developed remained above baseline levels. This was in contrast to results from a chronic oral ingestion procedure that exposed rats to caffeine evenly across each 24 h cycle, which resulted in evidence for a complete and insurmountable tolerance (12,15).

TABLE 2

$AUC_{0-4\ h}$ OF ACTIVITY, CAFFEINE, AND THREE METABOLITES FOR DAILY CHRONIC IP CAFFEINE 20 mg/kg DOSES IN RATS

Day	Activity Counts × h	Caffeine	Theobromine	Paraxanthine	Theophylline
			($\mu g \times h/ml$)		
1	34568.18 (±13746.00)	74.60 (±4.47)	1.91 (±0.50)	2.63 (±0.78)	1.37 (±0.34)
2	23791.43 (±7642.22)	70.71 (±2.52)	5.27 (±0.40)	4.18 (±0.71)	2.71 (±0.30)
3	23586.63 (±8095.11)	83.04 (±4.22)	4.46 (±0.42)	4.08 (±1.0)	2.78 (±0.32)
6	15360.78 (±3907.56)	78.04 (±3.12)	5.35 (±0.49)	4.89 (±1.30)	2.84 (±0.35)
11	13574.25 (±7124.47)	81.97* (±5.43)	3.29* (±0.13)	3.17* (±0.17)	3.58* (±0.11)
21	12894.07 (±3704.31)	82.56* (±4.06)	3.30* (±0.78)	3.47* (±0.69)	3.85* (±0.78)

*Unpublished data.

In the present study, locomotor activity rate was somewhat variable in the early phases of chronic exposure to caffeine as shown by the sizes of the standard errors (Figs. 2 and 4). However, the standard errors became much smaller as chronic administration progressed and tolerance had developed. Individual differences in the rate at which tolerance to the effects of caffeine developed contributed to the high, initial variances.

Brain concentrations consistently reflected plasma concentrations over a 4 h course in mice after IP injections with 20 or 40 mg/kg caffeine (18). The maximal locomotor stimulant activity occurred in a brain-concentration range of 10–20 $\mu g/g$, while lower and higher concentrations produced either no additional stimulation or decrements in activity. A plasma caffeine-concentration dependency also occurred for rats in a caffeine discrimination task (24). Both plasma caffeine level and caffeine-appropriate discriminative responding showed a rapid and parallel increase after a 32 mg/kg IP caffeine dose, followed by a slow decline in the plasma caffeine concentration that was accompanied by a rapid decline in caffeine discrimination, a differential effect suggesting the development of acute tolerance.

In the present study, for all IP caffeine doses there were clockwise hysteresis effects between serum caffeine level and the resulting locomotor activity rate, indicating the develop-

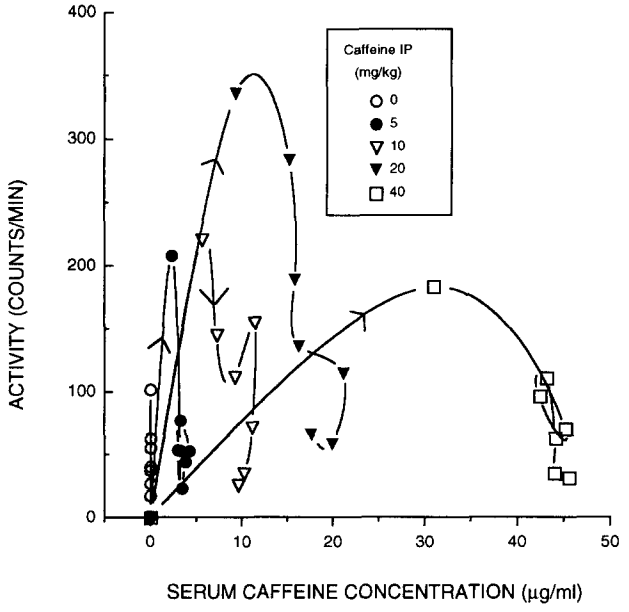

FIG. 7. Spline curve plot for activity rates as a function of serum caffeine concentration for four IP doses. Arrows indicate the direction of time flow.

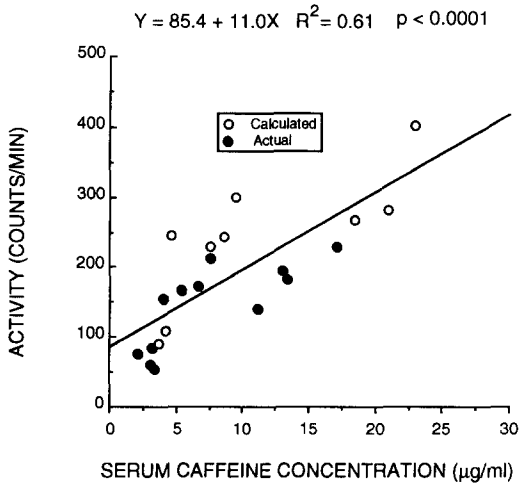

FIG. 8. Mean locomotor activity rates during 4 h sessions as a function of serum caffeine levels during sessions. Serum caffeine levels were produced by presession 3 h schedule-induced oral caffeine self-administration at three caffeine dose levels. The open circles were the calculated serum levels at 0.25, 0.5, and 0.75 h using the formula described in the Method section.

ment of acute tolerance (Fig. 7). Furthermore, within each dose level administered, peak serum caffeine was associated with a lowered locomotor activity rate (Figs. 5A and 1). Comparing across administered dose levels, serum caffeine concentration did not predict locomotor activity rate. For example, after the 20 mg/kg IP caffeine dose, which produced a serum caffeine concentration of 9.3 μg/ml, the locomotor activity rate was 335 counts/min. But after the 10 mg/kg dose, a similar serum caffeine level of 9.2 μg/ml produced 111 counts/min, and as it passed through a serum peak level (11.4 μg/ml) and descended to reach a similar level again (9.5 μg/ml) it produced a locomotor activity rate of only 26 counts/min (Fig. 7). The decreasing effect over time produced by the same serum caffeine concentration is indicated in the figure by the phenomenon of clockwise hysteresis. The development of acute tolerance occurs in humans to the pressor effects of caffeine (29).

In contrast to the results from IP administration, by the end of the 3 h oral self-administration session, caffeine concentrations in serum presumbly were well equilibrated with receptor-site concentration so that locomotor activity was a linear function of serum caffeine concentration (Fig. 8). Therefore, the peak concentration (19 μg/ml) for the highest orally self-administered caffeine dose (37.9 mg/kg) produced the highest activity rate.

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 (8). These investigators 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. In the present study, locomotor $AUCs_{activity(0-4\ h)}$ decreased as the combined $AUCs_{(0-4\ h)}$ for caffeine and the three active metabolites increased over days with daily 20 mg/kg IP injection (Table 2). Inasmuch as the higher serum caffeine concentrations after acute IP caffeine doses were associated with decreased locomotor activity (Figs. 1 and 7), the decrease in $AUCs_{activity(0-4\ h)}$ that occurred as a function of the increasing combined $AUCs_{(0-4\ h)}$ for caffeine and the three metabolites during chronic IP caffeine dosing may be attributable to the same mechanism.

The present research attained experimental precision with the use of relatively few animals by using repeated-measure, within-subject designs for both the pharmacokinetic and locomotor activity studies. An incomplete tolerance to the effect of chronic exposure to caffeine by both the IP route and oral self-administration route occurred, with tolerance developing more rapidly by the IP route. The development of acute tolerance was evident in the clockwise hysteresis spline plots for all IP caffeine doses. In terms of the serum caffeine AUC (4 h) values, considerably greater locomotor activity occurred following the oral self-administration of caffeine, compared to the IP route. With oral self-administration of caffeine, serum caffeine concentration was a linear predictor of locomotor activity rate, and the function accurately estimated activity rate under the water-polydipsia condition (the y-intercept, Fig. 8).

ACKNOWLEDGEMENTS

This work was supported by Grants R01 DA 05305 and K05 DA00142 from the National Institute on Drug Abuse. The authors acknowledge the dedicated technical assistance of Fang Ma and L. Jin with respect to HPLC analyses.

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