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Dose-Dependent Surmountability of Locomotor Activity in Caffeine Tolerance

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LAU, C. E. AND J. L. FALK. *Dose-dependent surmountability of locomotor activity in caffeine tolerance.* PHARMACOL BIOCHEM BEHAV **52**(1) 139–143, 1995.—For two chronic intraperitoneal caffeine dose regimens (10 and 80 mg/kg per day), tolerance developed rapidly (2–3 days) to the stimulatory effects of caffeine on locomotor activity. However, surmountability of the tolerant activity rate levels by caffeine administration was dose dependent: Activity rate was restored fully by acute caffeine administration for the 10 mg/kg per day series, but not for the 80 mg/kg per day series. The extent of tolerance was also dose-dependent: Tolerance was incomplete for the low-dose daily caffeine series but complete for the high-dose series. Upon discontinuation of daily caffeine dosing, activity rate decreased to the original baseline levels for both chronic series. Caffeine tolerance and the quantification of its surmountability may be explained by the pharmacokinetics of caffeine and the upregulation of adenosine receptors.

Caffeine tolerance Locomotor activity Surmountability Dimethylxanthines Caffeine pharmacokinetics

MARKED tolerance to the repeated administration of caffeine is known to develop in rats with respect to locomotor activity (14,18,24), caffeine discriminative-stimulus effects (14), and operant behavior (6,26), and in humans to the cardiovascular effects of caffeine (22). Mechanisms underlying tolerance to the increased locomotor activity produced by caffeine following chronic administration are not well understood. Caffeine and other methylxanthines (MXs) have antagonist actions at brain adenosine receptor sites (25,26). Tolerance to caffeine can be produced in rodents by allowing scheduled access to drinking water adulterated with different concentrations of caffeine, resulting in an intake of 10–220 mg/kg per day (1,7,13). It has been proposed that caffeine tolerance should be surmountable if it is due to the upregulation of adenosine receptors (15). However, locomotor activity did not increase as a function of acute caffeine dose (3–175 mg/kg) when rats were made tolerant by maintaining them at daily oral-intake doses higher than 36 mg/kg (13–15). On the other hand, the low locomotor activity level could be partially surmounted by acute caffeine administration when the orally self-administered caffeine doses were lower (10–20 mg/kg) (7,13,14). The degree of surmountability with respect to locomotor activity produced by test caffeine doses thus may be a function of the daily maintenance level of caffeine in rats. However, low locomotor activity was partially surmountable when test caffeine doses were given to caffeine-tolerant mice maintained at extremely high daily doses >200 mg/kg (1).

The locomotor activity produced by caffeine has a biphasic dose–response relation, with stimulation occurring at lower doses (5–10 mg/kg), greater activity at 10–50 mg/kg, and depressed activity at doses higher than 50 mg/kg in rats (14, 18,27). Caffeine is biotransformed to 28 metabolites that have been detected in the urine of animals and humans (3). Among them, two of the three dimethylxanthine (DMX) metabolites, paraxanthine and theophylline, also produced locomotor activity changes that were biphasic functions (20,27). Thus, DMXs probably contribute to changes in the locomotor activity produced by caffeine. The half-lives for caffeine, paraxanthine, theophylline, and theobromine were 2.8, 5, 10, and 14 h, respectively (19). Serum concentrations of caffeine and its three DMXs were still detectable after 31 h and the total concentration of DMXs (0.8 μg/ml) was much higher compared to caffeine (0.06 μg/ml) at 24 h after 10 mg/kg intraperitoneal (IP) caffeine because of the longer half-lives of DMXs than caffeine in food-limited rats (19). Furthermore, caffeine exhibited saturation (nonlinear) kinetics at about a 5 mg/kg caffeine dose administered to ad lib rats (2,17), whereas linear kinetics in the dose range of 10–40 mg/kg was found in food-limited rats (19). When saturation is approached, the rate of caffeine elimination becomes progressively slower, and small

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dose increments can cause disproportionately large increases in serum caffeine concentration. Cross-tolerance to the caffeine-induced stimulation of locomotor activity in caffeine-tolerant rats by theophylline (9) lends further support to the participation of these DMXs in the expression of the chronic effects produced by caffeine. Thus, it is important to consider total MXs, rather than caffeine alone, to delineate the effect of caffeine administration under both acute and chronic dose regimens.

The aim of the present study was to investigate the effects of IP caffeine doses (5–120 mg/kg) on locomotor activity using a within-group design under a food-limited condition with caffeine-tolerant rats maintained under two different dose regimens by the IP route: a low-dose series (10 mg/kg per day) and a high-dose series (80 mg/kg per day). These two chronic series were separated by 2 weeks, because it has been reported that stimulation of locomotor activity by caffeine was fully restored within 2–3 weeks after removal of caffeine from the drinking water (13). The rationale for using these two dose regimens derives from the biphasic effect of caffeine on locomotor activity—a low dose from the ascending limb and a high dose from the decending limb. It is interesting to apply a knowledge of caffeine pharmacokinetics to examine the surmountability of caffeine tolerance with respect to locomotor activity in a dose-dependent fashion.

METHOD

Animals

Four adult male, albino rats of the Holtzman strain (Madison, WI) with a mean initial body weight of 383 g (range 380–388) were housed individually in stainless-steel cages in a temperature-regulated room with a 12L : 12D 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 wt. Doses were calculated as the base.

Apparatus

Spontaneous locomotor activity was measured, as described previously (18), 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) that 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.

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, 2-h activity-monitoring sessions were conducted daily (7 days/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. Executing activity sessions overnight permitted a quiet environment isolated from the noise of routines performed in the room during the day. At 0900 h, animals were returned to their home cages and given food rations to maintain their criterion weights. This 15-h delay procedure was included to ensure that no prefeeding increase in activity would occur toward the end of the session, which could have confounded the evaluation of drug-induced changes in activity. After a month of daily activity measurement, all animals displayed low intersession variability and drug administration commenced.

Daily vehicle IP injections were given to animals immediately before they were placed into their individual activity-monitoring cages. These vehicle injections served as a control for chronic caffeine doses. It required 1–4 vehicle injections for animals to return to their respective baselines. Immediately after the last vehicle injection, animals received chronic daily IP injections of 10 mg/kg for 39–43 days. Each animal then received test 5, 20, 40, 80, and 120 mg/kg caffeine doses in an ascending dose order at 3–5 day intervals in place of the 10 mg/kg dose after a second locomotor activity baseline was established (range 10–12 days) under the daily 10-mg/kg caffeine dose regimen. Rats 2 and 3 did not received the 120-mg/kg caffeine dose during this chronic series. After completion of the dose–response curves and when the daily locomotor activity rate was stable, daily doses of caffeine were terminated and vehicle was administered for three consecutive sessions.

Two weeks after the first chronic caffeine series, animals received one to three vehicle injections as in the first series, followed by two daily doses (a.m. and p.m. doses) of 40 mg/kg IP caffeine, a total of 80 mg/kg per day, for 22–38 days. The first caffeine dose was given immediately before the activity session (p.m. dose) and the second caffeine dose (a.m. dose) was given at 0900 h immediately before animals were returned to their home cages. The maintenance p.m. dose was 40 mg/kg except on the days animals received 5, 20, 40, 80, or 120 mg/kg caffeine as in the first chronic caffeine series. Rats 1 and 4 did not receive the 120-mg/kg caffeine dose.

RESULTS

Figure 1 (leftmost points) shows, for each animal, the 2-h locomotor activity rate for the four daily sessions immediately preceding the two chronic daily series of IP 10 and 40 mg/kg caffeine administration. The first point of the chronic 80 mg/kg per day series shows the results of the initial 40 mg/kg p.m. dose. The ensuing points in the panel show the results of the combined 40 mg/kg a.m. and p.m. doses (80 mg/kg per day), as described in Method. There were marked increases in activity rates from baseline levels (B) for the initial caffeine doses (10 or 40 mg/kg) for the two chronic series. However, tolerance developed rapidly to these locomotor activity increases produced by caffeine after the 1st day for both series, and the rates decreased to tolerant baseline levels by the 2nd or 3rd day of dosing for all four animals.

The center panel of Fig. 1 shows the dose–response curves for caffeine during the two chronic series. Activity rate was a

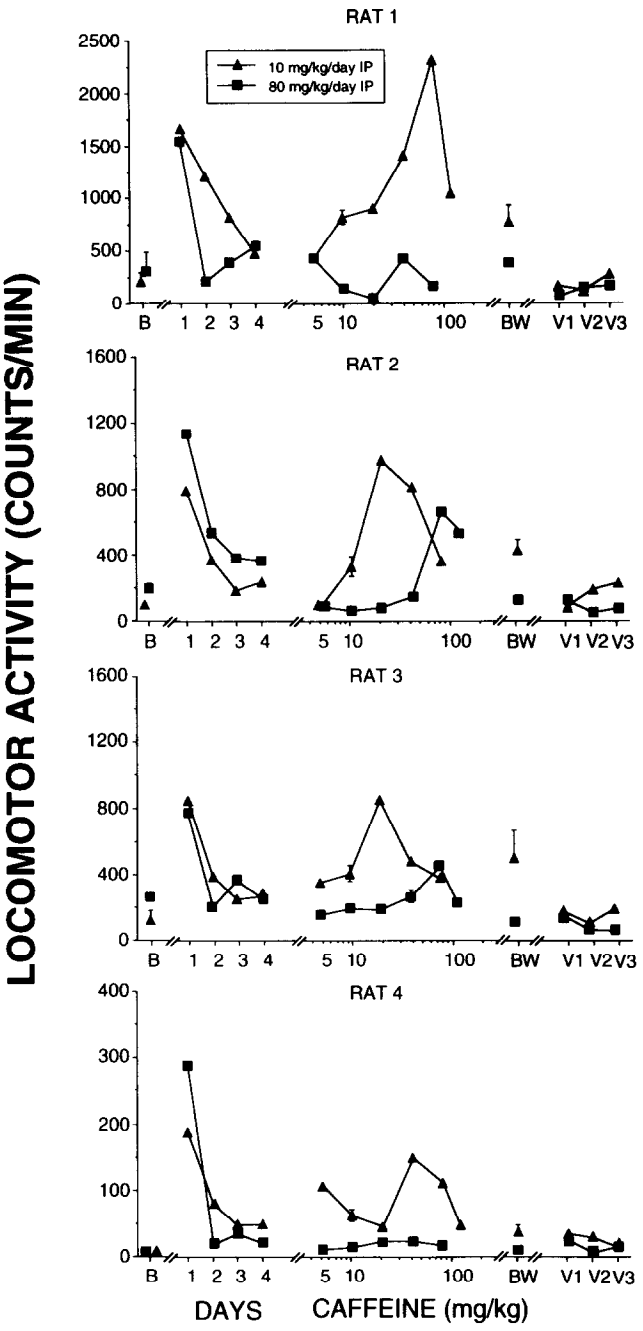

FIG. 1. Effects of caffeine on 2-h locomotor activity rates for two chronic caffeine dose regimens in four rats. Left panel: activity rate as a function of daily dose and days. Center panel: activity rate as a function of acute IP caffeine test doses during the two chronic caffeine series. Right panel: effects of vehicle injections after caffeine withdrawal. (B) Baseline activity rates (3-day means ± SE before each chronic-caffeine series). (BW) Before-withdrawal activity rates (means ± SE of last 3 days of caffeine dosing). (V) Vehicle injection.

biphasic function of caffeine doses. For the chronic 10 mg/kg per day caffeine series, the activity-rate decrease was surmountable with increasing doses of caffeine: between 40 and 80 mg/kg (rat 1), between 10 and 40 mg/kg (rat 2), 20 mg/kg (rat 3), and 40 mg/kg (rat 4). For the 80 mg/kg per day series, rat 2 showed partial surmountability at the two highest caffeine doses. There was no evidence of surmountability for the remaining rats. Activity rates for the maintenance doses 10 and 40 mg/kg (Fig. 1) are the grand means ± SE of each 2-day mean before the caffeine doses that differed from the maintenance dose. These values were similar to day 4 and the values for the mean of the last 3 days before caffeine withdrawal (points BW). The BW values, before withdrawal, show the baseline tolerant activity rates during both chronic caffeine series. However, BW values were higher than baseline (B) values for the 10 mg/kg per day series for all four animals, which indicates that tolerance was not complete, but for the 80 mg/kg per day series, the BW values were not different from the B values, indicating complete tolerance. Upon discontinuation of the daily caffeine doses, activity rate decreased to the original baseline levels as indicated on the rightmost panel (vehicle points) for each animal.

DISCUSSION

The rate and extent of tolerance to the stimulating effect produced by caffeine on locomotor activity in rats vary across studies. Caffeine tolerance in food-limited rats developed rapidly and was incomplete by the IP route (20 mg/kg per day), whereas tolerance only developed after about 2 weeks of exposure to caffeine by schedule-induced oral self-administration (36.5 mg/kg per day) and tolerance was also incomplete (18). However, caffeine tolerance was complete and insurmountable in ad lib rats administered drug with scheduled access to caffeine drinking solution if the dose was higher than 36 mg/kg per day (14). In the present study, the extent of tolerance was dose dependent: Tolerance was incomplete for the low-dose daily caffeine series (10 mg/kg per day) but complete for the high-dose series (80 mg/kg per day). Thus, various factors can influence the rate and extent of the development of caffeine tolerance. These factors can be summarized as route and schedule of caffeine administration, daily maintenance dose, and feeding conditions (deprived or ad lib). These factors are all important determinants of the metabolism and pharmacokinetics of caffeine. The present study, based on our previous pharmacokinetic data for caffeine and its three DMXs (18,19), was designed to determine whether the tolerance to the increased level of locomotor activity that resulted from chronic caffeine administration is surmountable. Acute caffeine administration produced a dose-dependent, biphasic relation with respect to locomotor activity, with stimulation at low doses and depression at high doses (14,18,27). This dose-dependent relation may have implications for the study of the phenomenon of caffeine tolerance. Tolerance surmountability may be a function of the maintenance dosing level used to produce the tolerance. If active metabolites are formed after the administration of a parent compound, the pharmacologic effect may correlate better with the summed concentrations of the parent compound and its active metabolites than with parent drug concentration alone (16). In rats, the three DMX metabolites are present in similar amounts (21) and are all pharmacologically active (6,8). Thus, evaluation of tolerance is made more difficult not only because of the biphasic nature of the caffeine dose–effect relation but also because of the increased total concentration of MXs during chronic caffeine administration. This may partially account for the decrease in activity that is interpreted as tolerance. In a previous study, daily IP caffeine 20-mg/kg doses maintained a high, constant serum area under the curve ($AUC_{(0\text{-}4\ h)}$) caffeine value, and caffeine was the major contributor to the total serum MX

$AUC_{(0\text{-}4\ h)}$ value. However, a twofold increase in the total DMX value was observed after the second caffeine administration and was maintained at that level for the 21 days of dosing (18). Also for food-limited rats, serum caffeine AUCs were proportional to IP caffeine doses (10–40 mg/kg) (19), whereas saturation kinetics have been found for ad lib rats administered doses <5 mg/kg and plasma concentrations of caffeine as low as 2 to 4 μg/ml (2,5,17). Saturation kinetics imply that caffeine will remain longer and lead to higher serum concentrations compared to the linear kinetic case. In humans, food deprivation or restriction can occur for cosmetic, health, or economic reasons. In animals, to study the effects of caffeine on behavior, a food-deprivation regimen is often used to implement a food-reinforced behavioral baseline. Inasmuch as there are differences between the caffeine pharmacokinetics of ad lib and food-limited animals, it was of interest to study the effects of caffeine on locomotor activity in food-deprived animals so that their results could be compared with operant behavior studies that use food-limited animals. Two distinct types of caffeine tolerance have been reported: surmountable, under schedule-controlled behavior, and insurmountable with spontaneous locomotor activity (14). However, the two types of tolerance may be the result of the food regimens used rather than the behaviors studied.

In the present study, the tolerant baseline activity, which was first established by 10–12 days of daily 10-mg/kg caffeine doses, was surmountable by higher test caffeine doses (20–40 mg/kg) for all four animals. As indicated earlier (18), daily 20 mg/kg per day IP doses produced a steady state that, even after 21 dosing days, yielded only about a 10% increase in the total $AUC_{(0\text{-}4\ h)}$ of MXs. Thus, in terms of pharmacokinetic accumulation of MXs, there was no ceiling effect that would prevent surmountability from occurring. It is generally recognized that the antagonism of adenosine receptors at least partly underlies the pharmacologic effects of low doses of caffeine, whereas phosphodiesterase inhibition and calcium mobilization become more significant at higher doses (8,12). Upregulation of adenosine receptors is considered to be one of the possible mechanisms mediating tolerance to caffeine. There was a 25% increase in adenosine receptor binding sites in rat cerebral cortical membranes after 7 days of IP caffeine at 20 mg/kg per day (10). For rat 1 (Fig. 1), the 80-mg/kg dose produced an activity rate that not only surmounted the chronic 10 mg/kg per day tolerant level, but was even greater than the first 10-mg/kg dose of the chronic series (Fig. 1, day 1). The caffeine doses (20–40 mg/kg) needed to restore the activity rate levels of the four animals were different. This may reflect individual differences with respect to pharmacokinetics of the MXs—for example, liver enzyme levels (23)—and the percent of adenosine receptor increases. Upregulation of adenosine receptors after chronic administration of caffeine was generally in the range of 10–30% in rats (4,10,11).

Despite high, acute caffeine doses during the 80 mg/kg per day caffeine chronic series, locomotor activity remained at tolerance-baseline levels for rats 1, 3, and 4. For rat 2, the 80- and 100-mg/kg test caffeine doses increased the activity rate, but the level never exceeded the 1st-day activity level produced by the 40-mg/kg IP caffeine dose for the series. The purpose of the a.m. caffeine doses for the series was to maintain high MX levels in the body. It is difficult to separate tolerance from overdose or toxicity when the caffeine doses used are from the descending limb of the biphasic relation.

We used the IP route rather than oral self-administration for maintaining chronic caffeine dose regimens to evaluate the surmountability of test caffeine doses on locomotor activity for two reasons. First, the pharmacokinetics of IP caffeine in food-limited rats has been studied extensively (18,19). Second, it has been demonstrated that the number of adenosine receptors in rat cerebral cortical membranes increased 25 and 28% after treatment with 20 mg/kg per day (7 days) and 75 mg/kg per day (12 days) IP caffeine doses, respectively (10,11). Inasmuch as these two dose levels produced similar percent increases in adenosine receptor number, it implies that a limited number of receptor sites can be upregulated. However, with low caffeine dose regimens (5–10 mg/kg per day) there were dose-related increases in adenosine receptors in rats (7). This limited increase in adenosine receptors further supports the chronic dose-level dependency of the phenomenon of surmountability of caffeine tolerance in locomotor activity. Caffeine acts as a competitive inhibitor for adenosine receptors in vitro (25,26). Upregulation of adenosine receptors has not been associated with changes in receptor affinity after chronic caffeine administration (7,10,11). Thus, caffeine may act as a noncompetitive, rather than a competitive, inhibitor during chronic caffeine administration.

Although there were individual differences in responsiveness to the effects of caffeine for the two chronic series in the present study, the results were consistent within each animal and comparable across animals. Complete restoration of locomotor activity levels by test doses of caffeine during the chronic low-dose series in these food-limited rats contrasted with the partial surmountability found for ad lib rats maintained on low caffeine dose regimens, 10–20 mg/kg (7,14). However, with high, chronic caffeine dose regimens (13–15) in such rats, and in the present study with food limitation, no evidence of surmountability was found.

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