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Tolerance to Behavioral Effects of Caffeine in Rats

STEPHEN G. HOLTZMAN AND IRA B. FINN

Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322

HOLTZMAN, S. G. AND I. B. FINN. *Tolerance to behavioral effects of caffeine in rats.* PHARMACOL BIOCHEM BEHAV **29**(2) 411–418, 1988.—Tolerance develops to three behavioral effects of caffeine in rats treated daily with the drug: stimulation of locomotor activity, rate-decreasing effect on food-reinforced operant responding, and discriminative stimulus effects. Tolerance induced by caffeine to stimulation of locomotor activity: (1) develops rapidly, being nearly maximal 24 hr after the start of daily treatment with 35–40 mg/kg in four divided oral doses; (2) is insurmountable (i.e., complete) so that rats are unresponsive, even to high doses of caffeine, and dose-response curves are displaced downward and flattened; (3) is pharmacologically specific, extending to other methylxanthines but not to nonxanthine psychomotor stimulants. Tolerance to the other two behavioral effects of caffeine: (1) appears to develop gradually; (2) is surmountable so that dose-response curves are displaced to the right; (3) extends to nonxanthine psychomotor stimulants, methylphenidate, in the case of discriminative effects. Thus, at least two distinct types of tolerance to behavioral effects of caffeine in the rat can be identified. The potency of an adenosine analog, R(−)-PIA, in depressing locomotor activity does not increase during or 24 hr after termination of chronic daily treatment with caffeine when rats are completely tolerant to caffeine-induced stimulation of locomotor activity. These results do not support the view that enhanced functional sensitivity of central adenosine systems (e.g., up-regulation of receptors) is the mechanism of tolerance to the stimulant effect of caffeine on locomotor activity.

Adenosine Caffeine Cross-tolerance Drug discrimination Locomotor activity Methylphenidate Methylxanthines Psychomotor stimulants Tolerance

DESPITE the fact that most caffeine consumption occurs on a chronic daily basis, there are surprisingly few reports in the clinical or preclinical literature on the behavioral consequences of chronic caffeine intake. In one of the first such studies in animals, Carney [6] tested caffeine in rats trained to press a lever under a 30-sec variable interval schedule of food reinforcement. Caffeine (32 mg/kg) was injected IP each day before the experimental session. After seven days tolerance developed to the drug-induced decreases in response rate and the caffeine dose-response curve was shifted to the right by a factor of six.

A more common means of administering caffeine chronically is to place the drug in the drinking water of the animals. After receiving 5.0–10 mg/kg/day of caffeine administered in this manner, rats were tolerant to the stimulant effect of caffeine on locomotor activity as evidenced by a 2-fold shift to the right of the caffeine dose-response curve [7]. Exposure of rats daily to higher doses of caffeine resulted in the development of complete tolerance to caffeine-induced stimulation of locomotor activity. Rats receiving 150–200 mg/kg per day of caffeine for two months by scheduled-access to water bottles containing drug solution showed no increase in activity during tests with 6.25–100 mg/kg of caffeine [14]. However, they responded to amphetamine with an increase in locomotor activity comparable to that of a drug-free control group. Thus, failure to respond to caffeine was not a consequence of debilitation or other impairments of motor function resulting from the chronic drug treatment. Similar findings were obtained in mice consuming an average of more than 200 mg/kg per day of caffeine for two weeks [1]. Full sensitivity to the stimulant effect of caffeine in the rat returned within 2–3 weeks after termination of daily caffeine administration [14].

From the foregoing it is apparent that tolerance develops to behavioral effects of caffeine in rats and mice. In the case of stimulation of locomotor activity, tolerance can be complete, pharmacologically specific, and reversible.

CHARACTERISTICS OF TOLERANCE TO CAFFEINE-INDUCED STIMULATION OF LOCOMOTOR ACTIVITY

Dosage and Temporal Parameters

Other characteristics of caffeine tolerance are just beginning to be revealed. We recently evaluated the extent of tolerance development to caffeine-induced stimulation of locomotor activity in the rat as a function of the dose of caffeine consumed daily [9]. Four groups of adult male rats were given scheduled-access [10], for 10 min every 6 hours, to water bottles containing either 0.25, 0.5, or 1.0 mg/ml of caffeine solution or drug-free tap water (control). These bottles were the animals' only source of fluid. Daily intake of caffeine averaged 19±1, 36±2, and 67±2 mg/kg (mean± SEM) for the three groups receiving the caffeine solutions. Dose-response curves for caffeine were determined during weeks 3–5 of chronic drug treatment, and are shown in Fig. 1. The locomotor activity of animals in the control group was increased significantly by 10 mg/kg of caffeine. In contrast,

rats drinking either 0.5 or 1.0 mg/ml solutions of caffeine were completely unresponsive to doses of caffeine as high as 175 mg/kg. Only rats consuming the 0.25 mg/ml caffeine solution had increased activity after some of the caffeine doses tested, i.e., 17.5–56 mg/kg, but the effects were small. In this group, too, the tolerance was not of the surmountable type. The caffeine dose-response curve was displaced downward and effects of the magnitude produced in the control group could not be attained by increasing the dose of caffeine being tested.

Thus, as little as 35–40 mg/kg/day of caffeine is sufficient to induce complete tolerance to the stimulant effect of the drug on the locomotor activity of rats. Moreover, this stimulant action of caffeine is attenuated markedly by daily caffeine doses of under 20 mg/kg.

The time course for development and loss of tolerance to caffeine-induced stimulation of locomotor activity in the rat is relatively short. Significant tolerance is evident after only 24 hr of scheduled-access to 0.5 mg/ml of caffeine solution and tolerance is largely gone by 48–72 hr after daily caffeine treatment is halted [9]. The temporal parameters and insurmountability of this caffeine tolerance resemble more closely a classical tachyphylaxis rather than tolerance due to pharmacokinetic factors or postsynaptic cellular adaptation [18].

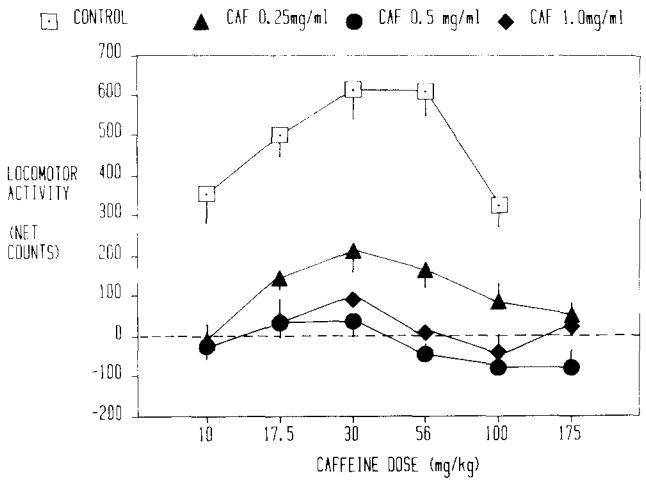

FIG. 1. Tolerance to caffeine-induced stimulation of locomotor activity in the rat as a function of the chronic daily dosage of caffeine. Determination of dose-response curves began three weeks after the start of scheduled-access to water bottles containing either drug-free tap water (control) or one of the three indicated concentrations of caffeine solution. Locomotor activity was recorded for 30 min beginning 35 min after a test dose of caffeine (10–175 mg/kg) was administered by gavage. Caffeine doses were tested twice weekly for effects on locomotor activity and were administered in random sequence to the animals in each group. Each point represents a mean and 1 SEM of one observation in each of 18 (control group) or 10 rats (chronic caffeine groups). Locomotor activity is shown as the number of activity counts following a test drug minus the number of activity counts during baseline sessions (i.e., net counts). Data are modified from [9].

Cross-Tolerance

Animals that are tolerant to an effect of a particular drug may also exhibit tolerance to that effect of other drugs, usually from the same or closely related pharmacologic class. The occurrence of cross-tolerance between drugs often is indicative of common mechanisms of drug actions. Therefore, we conducted a series of experiments to assess the pharmacologic selectivity of tolerance to caffeine-induced stimulation of locomotor activity.

Male rats of Sprague-Dawley descent, weighing 300–350 g, were given scheduled-access to water bottles containing 0.5 mg/ml of caffeine solution or, in some cases, 1.0 mg/ml. Control groups received scheduled-access to drug-free tap water. Dose-response relationships were determined for a variety of xanthine and nonxanthine behavioral stimulants, beginning at least 10 days after the start of chronic caffeine administration.

Results are summarized in Table 1. Rats consuming the 0.5 mg/ml solution of caffeine (approximately 35–40 mg/kg/day of caffeine) were completely tolerant of caffeine-induced stimulation of locomotor activity and were cross-tolerant to the stimulation of locomotor activity induced by two methylxanthine derivatives, theophylline and 7-(2-chloroethyl)theophylline. Cross-tolerance to stimulation of locomotor activity induced by 7-(2-chlorethyl)theophylline was also complete in rats consuming 0.5 mg/ml of caffeine solution. In fact, the dose of 7-(2-chloroethyl)theophylline producing the peak increase in locomotor activity in the control group, 30 mg/kg, actually decreased the locomotor activity of the group receiving caffeine chronically. Cross-tolerance to theophylline was graded with respect to the daily caffeine dosage. The stimulant effect of theophylline was diminished significantly in rats consuming 0.5 mg/ml of caffeine solution and was completely absent in rats consuming the 1.0 mg/ml solution (approximately 65–70 mg/kg/day of caffeine).

In contrast, the magnitude of stimulation of locomotor activity induced by nonxanthine behavioral stimulants was not modified significantly by chronic treatment with caffeine, even at 65–70 mg/kg/day (Table 1). This conclusion, which is based upon comparisons of peak drug effects, is also supported by the outcomes of comparisons of the full dose-response curves by analysis of variance.

The results of these experiments indicate that caffeine-induced tolerance to stimulation of locomotor activity in the rat is a phenomenon that is unique to the class of methylxanthines and is not shared by nonxanthine behavioral stimlants.

EXPERIMENT 1: EFFECTS OF CAFFEINE AND R(−)-PIA ON LOCOMOTOR ACTIVITY DURING CHRONIC CAFFEINE TREATMENT AND WITHDRAWAL

Caffeine and other methylxanthines are competitive inhibitors of adenosine binding sites in brain with relative affinities that correlate with relative potencies for stimulating locomotor activity of mice [8,26]. These observations have focused attention on brain adenosine systems as the substrates likely to be mediating the behavioral stimulant properties of caffeine. Indeed, analogs of adenosine depress the locomotor activity and operant responding of rodents, and these behavioral depressant effects are antagonized in an orderly manner by caffeine [3, 11, 12, 19].

Changes in the number of adenosine binding sites in the brain of animals receiving caffeine chronically are consistent with a role in the etiology of caffeine tolerance. Specific binding of [^{3}H]cyclohexyladenosine was increased 25–50% in the brain of mice consuming approximately 100 mg/kg/day of caffeine admixed with food for 2–4 weeks [5]. Similarly, both the binding of [^{3}H]phenylisopropyladenosine and [^{3}H]diethylphenylxanthine was doubled in the brain of mice that had

TABLE 1

PHARMACOLOGIC SPECIFICITY OF TOLERANCE TO CAFFEINE-INDUCED STIMULATION OF LOCOMOTOR ACTIVITY[a]

Drug	Dose Range Tested (mg/kg)	Dose (mg/kg)	Peak Effect Locomotor Activity (net counts) Control	Caffeine (0.5 mg/ml)	Caffeine (1.0 mg/ml)
Caffeine	3.0–100	30	446±62	−2±75‡	—[b]
Theophylline	3.0–100	30	397±98	119±67*	56±45†
7-(2-chloroethyl)-theophylline	1.0–30	30	184±61	−123±45‡	—
d-Amphetamine	0.1–3.0	1.0	762±140	775±128	—
Cocaine	1.0–30	30	471±146	491±185	478±94
Diethylpropion	1.0–30	10	793±202	483±125	622±118
Mazindol	0.3–10	3.0	520±173	308±141	369±75
Methylphenidate	0.3–30	10	975±221	886±68	—
Phendimetrazine	1.0–100	100	991±200	509±138	—
Phentermine	1.0–30	10	551±107	303±157	—

[a]Separate groups of rats (n=9/group) received scheduled-access to either drug-free tap water (control) or 0.5 or 1.0 mg/ml of caffeine solution. The indicated drugs were tested for effects on locomotor activity in two 30-min sessions per week; each session began 35 min after IP administration. Locomotor activity is shown for the dose of each test drug that produced the biggest increase and represents the number of activity counts following the test drug minus the number of activity counts during base line sessions (i.e., net counts). Each value is a mean±SEM (n=9).

[b]Not tested in this group.

Differs from corresponding control (two-tailed Student's *t*-test): *$p<0.05$; †$p<0.01$; ‡$p<0.001$.

consumed in excess of 200 mg/kg/day of caffeine in drinking water for two weeks [1]. As little as 10 mg/kg/day of caffeine for 14 days was associated with a 25% increase in [^{3}H]cyclohexyladenosine binding sites in rat mesencephalic reticular membranes [7]. It may be recalled that in the latter two studies this up-regulation of binding sites was associated with tolerance to caffeine-induced stimulation of locomotor activity *(vida supra)*. In addition, R(−)-N^6(2-phenylisopropyl)adenosine [R(−)-PIA] was more potent, by 3–10-fold, in depressing the locomotor activity of mice receiving caffeine chronically as compared to control animals [1]. That the hypotensive effect of intravenously infused adenosine in rats was potentiated 24 hr after the termination of chronic caffeine treatment (approximately 65 mg/kg/day for three weeks) also suggests a functional role for the increased adenosine binding sites [27].

In order to study further possible increases in the sensitivity of central adenosine systems that might be induced by prolonged exposure to caffeine, we compared the effects of caffeine and R(−)-PIA on locomotor activity during and 24 hr following termination of chronic caffeine treatment. Rats withdrawn for 24 hr from chronic treatment with caffeine have significantly reduced spontaneous locomotor activity [9,14] and increased sensitivity to the hypotensive effect of adenosine [27]. Hence, alterations in the sensitivity of neuronal substrates mediating caffeine tolerance should be apparent after 24 hr of withdrawal.

METHOD

The subjects were male rats of Sprague-Dawley descent (Sasco, Inc., Omaha, NE) weighing approximately 300 g at the time that they were received from the supplier. Each animal was housed individually in a polycarbonate cage maintained in a ventilated cabinet designed to control the access of the animal to its water bottle [9]. Food (Purina Rat Chow) was available continuously in the home cage. The cabinet was illuminated between 7:00 a.m. and 7:00 p.m.

Caffeine was administered chronically to the rats by the method of scheduled access to water bottles containing 0.5 mg/ml of caffeine (anhydrous base) dissolved in tap water. Animals in the control group received scheduled access to drug-free tap water. The bottles were placed in a holder positioned above the cage of each animal. A motor mounted on the top of the cabinet and controlled by an electronic timer rotated the bottle holders thus bringing the drinking spouts into and out of the cages. The drinking spouts contained ball bearings to minimize fluid spillage.

Animals were given 10-min access to their water bottle, which was their only source of fluid, every 6 hr, beginning at either 9:00 or 11:00 a.m. Daily caffeine intake was monitored periodically by weighing bottles on consecutive days after the 9:00 or 11:00 a.m. access. A difference of 1.0 g in bottle weight was assumed to be equivalent to 1.0 ml of solution consumed after correcting for spillage of 3.0 ml/day. The daily intake of caffeine averaged 40 mg/kg over the course of this experiment.

Locomotor activity was measured with six 2-channel Electronic Activity Monitors (31404, Stoelting Co., Chicago, IL). Each rat was placed in a polycarbonate rat cage (51×41×22 cm) centered on a sensor platform (SA1566, Stoelting Co.). The cage and sensor were housed in a ventilated sound-attenuated chamber that was illuminated by a small fluorescent bulb. The detection threshold of each sen-

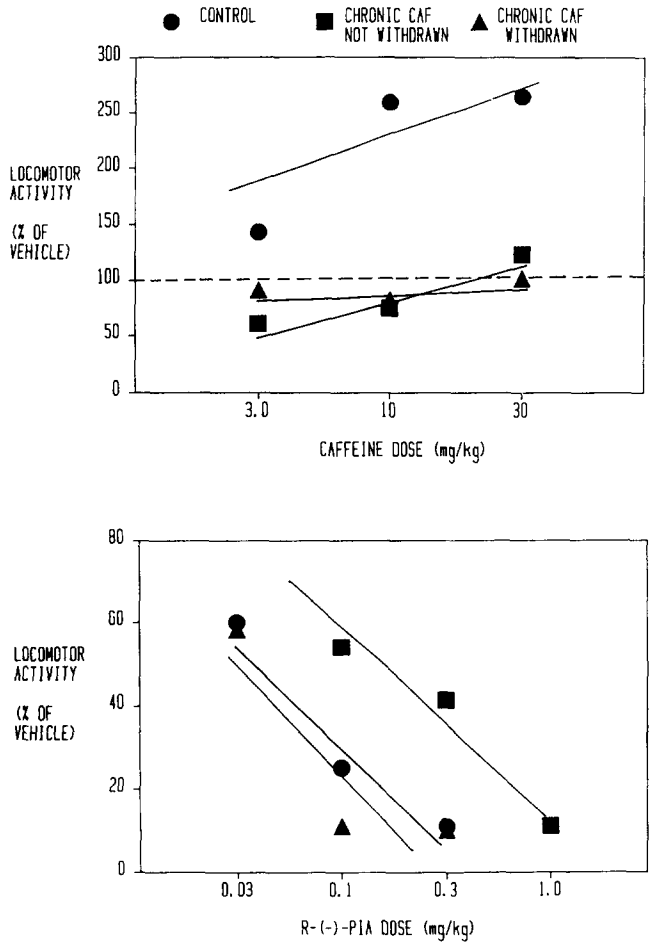

FIG. 2. Effects of caffeine (top panel) and R(−)-PIA (bottom panel) on the locomotor activity of control rats, rats receiving uninterrupted chronic daily treatment with caffeine, and rats withdrawn for 24 hr from chronic daily treatment with caffeine. Locomotor activity is expressed as a percent of activity recorded after the administration of vehicle for caffeine or R(−)-PIA. Each point is a mean based upon one observation in each of nine rats. The straight lines represent linear regressions fitted to the points by the method of least squares.

sor was calibrated with a swinging pendulum to measure gross movement in the horizontal plane corresponding to the locomotor activity of the animals. Activity counts were recorded by a microcomputer.

All rats were habituated to handling and injection procedures and to the activity chambers for 5 days before drug testing began. Drugs were administered IP 30 min before a rat was placed into an activity chamber, which was 35 min before the start of the experimental session. Following a 5-min habituation period during which activity was not recorded, locomotor activity was measured for 30 min. Animals in the control group and animals receiving caffeine chronically and not withdrawn were tested twice weekly at 3–4 day intervals. Animals in the withdrawal group were tested once weekly, 24 hr after the caffeine solution in their water bottle was replaced with drug-free tap water.

Caffeine sodium benzoate was dissolved in 0.9 percent NaCl and R(−)-N^6-(2-phenylisopropyl)adenosine was dissolved in a minimal volume of 0.1 N HCl and brought to final volume with 0.9% NaCl. Doses of each drug were administered to the rats in a random sequence that also included the drug vehicle.

RESULTS AND DISCUSSION

Caffeine was tested at doses of 3.0, 10 and 30 mg/kg, which defines the ascending limb of the dose-response curve for caffeine-induced stimulation of locomotor activity in control animals. Consistent with previous observations (i.e., Fig. 1), these doses of caffeine had no effect on the locomotor activity of rats receiving chronic daily treatment with caffeine (Fig. 2). Rats withdrawn for 24 hr from chronic treatment with caffeine also were completely unresponsive to the test doses of caffeine (Fig. 2). Thus, complete tolerance to the stimulant effect of caffeine on locomotor activity persists for at least 24 hr after withdrawal of the drug.

R(−)-PIA, 0.03–0.3 mg/kg, produced a dose-dependent decrease in the locomotor activity of rats in the control group (Fig. 2), consistent with the reports of others. R(−)-PIA also decreased, dose-dependently, the locomotor activity of rats receiving uninterrupted chronic daily treatment with caffeine. However, the PIA dose-response curve in this group was shifted to the right of the curve in the control group by a factor of 3 (Fig. 2), consistent with a competitive interaction between caffeine and R(−)-PIA at the receptor level. Thus, the adenosine-antagonist properties of caffeine remain apparent even in animals that are completely tolerant to the stimulant effect of caffeine on locomotor activity.

The dose-response curve for R(−)-PIA in rats withdrawn for 24 hr from chronic daily treatment with caffeine was similar to the curve for the control group. It is conceivable that 24 hr after the last dose of caffeine there is sufficient drug remaining in the brain to antagonize, at least weakly, the depressant effect of R(−)-PIA, thereby masking evidence of increases in functional sensitivity of adenosine systems. On the other hand, the enhanced hypotensive effect of adenosine was apparent in rats withdrawn for 24 hr from a daily dose of caffeine higher than the 40 mg/kg used in the present study [27].

Our results provide no indication that chronic treatment with caffeine enhances the behavioral depressant effects of R(−)-PIA at time when complete tolerance to caffeine-induced stimulation of locomotor activity is still very much in evidence. These results contrast with the findings of Ahlijanian and Takemori [1]. This discrepancy is difficult to reconcile but may be attributable to any or several of the major differences between the two studies, such as testing procedure, dosage of caffeine administered daily, and animal species.

EXPERIMENT 2: TOLERANCE TO EFFECTS OF CAFFEINE ON SCHEDULE-CONTROLLED BEHAVIOR

The decreases in spontaneous locomotor activity of rats the day after cessation of chronic daily treatment with caffeine is suggestive of a drug withdrawal phenomenon. Drug withdrawal changes usually occur in a direction opposite to that produced by the drug when it is initially administered to the otherwise drug-free subject. Changes in schedule-controlled behaviors have proven to be exceptionally sensitive indicators of drug withdrawal with dependence-producing drugs of other pharmacologic classes, such as opiates [17] and benzodiazepines [21]. Rats pressing a lever

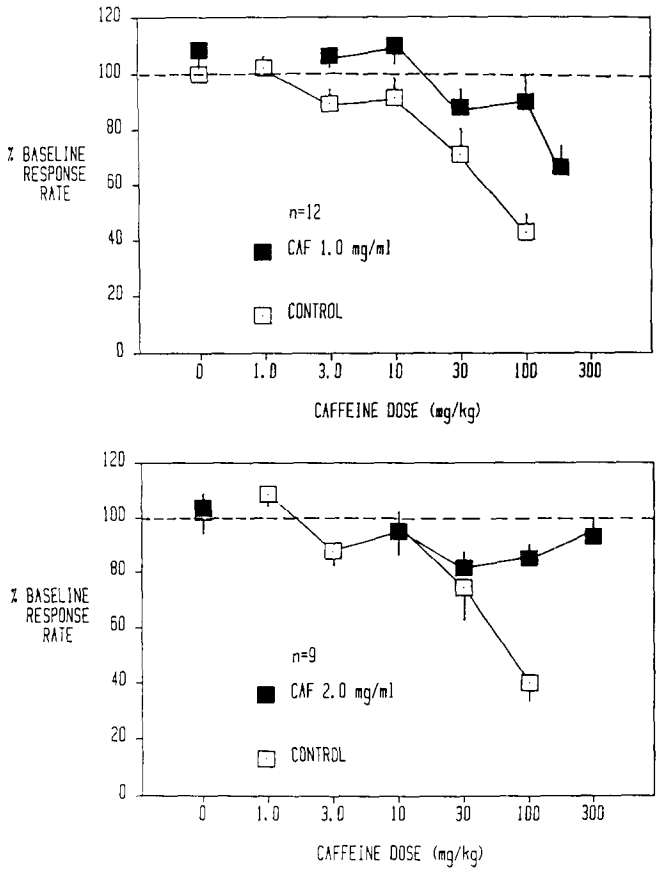

FIG. 3. Tolerance develops to the rate-decreasing effect of caffeine on responding maintained by a 1-min variable-interval schedule of food reinforcement. The extent of tolerance development is a function of the dosage of caffeine administered on a chronic daily basis. Dose-response curves were determined sequentially in the same set of rats during scheduled-access to water bottles containing first drug-free tap water (control), then 1.0 mg/ml of caffeine solution (top panel), and last, 2.0 mg/ml of caffeine solution (bottom panel). Each point represents a mean and 1 SEM based upon one observation in each of 12 or 9 rats. Response rate is expressed as a percent of the average rate of responding in baseline sessions during each stage of the study as follows: 1,893±315 responses per hour (control); 2,208±423 responses per hour (1.0 mg/ml caffeine solution); 1,777±362 responses per hour (2.0 mg/ml caffeine solution).

under a 30 sec variable interval schedule of food reinforcement had a 50% decrease in response rate on the first day that saline was injected in place of a daily dose of 32 mg/kg of caffeine [6]. In the following experiments we used a baseline of food-reinforced responding to study tolerance to caffeine and possible withdrawal changes upon abrupt termination of chronic drug treatment. These parameters were examined in rats maintained on two different daily dosages of caffeine by scheduled-access to water bottles containing caffeine solution.

METHOD

The subjects were male rats of Sprague-Dawley descent (Sasco, Inc., Omaha, NE) weighing initially 300–350 g and housed as described in experiment 1.

Animals were given access to their water bottle, which was their only source of fluid, for 10 min every 6 hr, beginning at 8:00 a.m. Depending upon the phase of the experiment, the water bottles contained either drug-free tap water or 1.0 or 2.0 mg/ml of caffeine solution. Daily caffeine intake was monitored by weighing the water bottles on consecutive days after the 8:00 a.m. access period. A difference of 1.0 g in bottle weight was assumed to represent consumption of 1.0 ml of solution after correcting for spillage of 4.0 ml per day. Caffeine solutions of 1.0 and 2.0 mg/ml resulted in daily caffeine intake of 50 and 90 mg/kg, respectively.

Experimental sessions were conducted in modular test chambers (Coulbourn Instruments, Lehigh Valley, PA), housed in sound-attenuating enclosures that were light proof and ventilated. Each test chamber contained a house light, a response lever, and a food-pellet dispenser and receptacle. A microcomputer controlled experimental contingencies and recorded data.

The rats were reduced to 80 percent of their normal body weight by restricted feeding and were trained to press the lever in test chambers in order to receive a 45 mg food pellet (Bio-Serv., Inc., Frenchtown, NJ). After the lever-pressing response was acquired, a 1-min variable interval schedule was put into effect so that the first response emitted after a variable time interval averaging 1 min produced delivery of a food pellet. Experimental sessions were conducted once daily, Monday through Friday, each session lasting 60 min.

After four weeks of training under the terminal schedule, the response rate of the rats varied not more than ±10% from day to day. The initial caffeine dose-response curve was determined at this time. Various doses of caffeine sodium benzoate (calculated as the free base) were administered by gavage (1.0 ml/kg of body weight) 15 min before a session on Tuesdays and Fridays. Doses of caffeine were administered to each animal in a different random sequence. Upon completion of the initial caffeine dose-response curve, the rats were given scheduled-access to 1.0 mg/ml of caffeine solution and the caffeine curve was redetermined beginning one week later. When the rats had received caffeine daily for one month, the drug solution in the water bottle was replaced with plain tap water and behavior was followed in daily experimental sessions for the next four days. The rats were then given scheduled-access to 2.0 mg/ml of caffeine solution and the series of tests was repeated.

RESULTS AND DISCUSSION

Before the start of chronic daily caffeine administration, 30 and 100 mg/kg of caffeine decreased the average rate of responding, the latter dose being approximately an ED50 (Fig. 3). These doses had little or no effect on responding when the rats were receiving caffeine chronically. A dose of 175 mg/kg of caffeine was required to decrease responding significantly in rats receiving the 1.0 mg/ml solution of caffeine daily, whereas a test dose of 300 mg/kg had no effect on responding during scheduled-access to 2.0 mg/ml of caffeine solution (Fig. 3). Higher doses of caffeine had serious adverse effects on the health of the animals and could not be tested.

Response rate did not change significantly on the four days after withdrawal from the 1.0 and 2.0 mg/ml solutions of caffeine. In the first instance, response rate ranged from 90±5 to 96±8 percent of baseline and in the second instance, 89±12 to 115±12 percent of baseline.

The rightward displacement of the caffeine dose-response curve, by a factor of approximately 5-fold, in rats maintained on the lower chronic daily dosage of caffeine (i.e., 50

TABLE 2

TOLERANCE TO THE DISCRIMINATIVE STIMULUS EFFECTS OF CAFFEINE AND CROSS-TOLERANCE WITH METHYLPHENIDATE

Test Drug	Daily Treatment†	ED_{50} (95% C.L.),* mg/kg First Curve	Second Curve
Caffeine	Caffeine (30 mg/kg)	5.0 (1.4–18)	20 (17–23)
Methylphenidate	Caffeine (30 mg/kg)	1.5 (1.0–2.1)	5.5 (3.1–9.5)
Caffeine	Methylphenidate (3.0 mg/kg)	5.2 (1.5–18)	15 (11–19)

*ED_{50}s and 95% confidence limits for caffeine-appropriate responding were determined by probit analysis of the results of tests of five or six doses in each of four rats. Data are adapted from [16].

†Injected IP twice daily for 3½ days between the first and second determination of the stimulus-generalization curve for the test drug indicated in the left-hand column.

mg/kg/day), is comparable in magnitude to that produced by Carney [6] with single daily injections of 32 mg/kg of caffeine. This outcome stands in contrast to that obtained with locomotor activity as the dependent variable where dose-response curves were displaced downward and complete and insurmountable tolerance was produced by lower daily doses of caffeine. It was not possible to determine if tolerance to the response rate-decreasing effect of caffeine produced by the higher chronic daily doses of caffeine (i.e., 90 mg/kg/day) is also surmountable. Drug toxicity imposed an upper limit on doses that could be tested safely.

The absence of changes in response rate following withdrawal from caffeine is somewhat puzzling in light of changes in locomotor activity that occur in rats withdrawn from caffeine. For example, the spontaneous locomotor activity of rats that had been maintained on 65–70 mg/kg/day of caffeine decreased 40–50 percent at 24 and 48 hr after termination of chronic drug treatment [9]. The average daily doses of caffeine consumed by rats in the present study, 50 and 90 mg/kg, should have been adequate to result in changes in operant behavior, especially in view of the sensitivity of this type of baseline to disruption by drug withdrawal. Rats responding under a schedule of reinforcement similar to the one used in this study evidenced marked decreases in response rate 24 hr after the final daily dose of only 32 mg/kg/day of caffeine [6]. However, in that study, the daily dose of caffeine was administered in a single injection prior to the experimental session. Such a protocol sometimes gives rise to behavioral tolerance and dependence, phemomena that may be distinct from the tolerance and dependence that result from the specific pharmacodynamic properties of the drug (e.g., [2,13]). Changes in schedule-controlled behavior following termination of chronic treatment with caffeine have not occurred consistently with a variety of other schedules of food reinforcement (Finn and Holtzman, unpublished observation; J. Carney, personal communication). Postwithdrawal changes in locomotor activity seem to be relatively robust and reproducible in rats that had been treated chronically with at least 65–70 mg/kg/day of caffeine [9,14]. On the other hand, postwithdrawal changes in schedule-controlled behavior are far more difficult to demonstrate, even under conditions of caffeine treatment sufficient to induce tolerance to response-rate-decreasing effects of caffeine and to result in postwithdrawal depression of locomotor activity.

TOLERANCE TO DISCRIMINATIVE EFFECTS OF CAFFEINE AND CROSS-TOLERANCE WITH METHYLPHENIDATE

Drug discrimination paradigms afford a behavioral endpoint that can reflect the neuronal substrates with which a drug interacts to produce stimulus control of behavior [24]. Rats trained to discriminate between injections of saline and 10 mg/kg of caffeine emit the caffeine-appropriate response in tests of stimulus generalization to methylphenidate [15]. Phentolamine, an *alpha*-adrenergic receptor blocking drug, antagonizes dose-dependently and surmountably both the discriminative effects of caffeine and the caffeine-like discriminative effects of methylphenidate. Thus, the discriminative stimulus effects of caffeine and methylphenidate may have in common a noradrenergic component [15].

There is evidence that tolerance develops to the discriminative effects of caffeine in the rat [22,23], but the issue has not been addressed directly. Therefore, experiments were performed (a) to determine whether or not tolerance develops to the discriminative effects of caffeine, and (b) to evaluate further the possible commonalities in the discriminative effects of caffeine and methylphenidate by testing for the occurrence of cross-tolerance between the drugs [16].

Rats were trained to discriminate between IP injections of saline and 10 mg/kg of caffeine as described previously [15]. A stimulus-generalization curve was determined for caffeine or methylphenidate administered IP on the morning of day 1 using a cumulative dosing procedure [4,20]. The rats were then returned to their home cage where they were injected later on day 1 and twice on days 2, 3 and 4 with either 30 mg/kg of caffeine or 3.0 mg/kg of methylphenidate by the IP route. The stimulus-generalization curve was redetermined in the same animals on the morning of day 5.

The principal findings are summarized in Table 2. Tolerance developed to the discriminative stimulus effects of caffeine and cross-tolerance between caffeine and methylphenidate could be demonstrated. Twice daily injections of

30 mg/kg of caffeine for three and one-half days increased the ED_{50} for caffeine-appropriate responding by a factor of 4 for caffeine and by almost as much for methylphenidate. Conversely, twice daily injections of 3.0 mg/kg of methylphenidate increased the ED_{50} of caffeine by almost 3-fold. A greater degree of tolerance might have been demonstrated had the drugs been administered twice daily for a longer period of time. However, this must be weighed against the risk that drug cues will lose their saliency and stimulus control of behavior will diminish if animals remain untested for lengthy periods while being exposed to drugs in a novel context. The symmetrical cross-tolerance between caffeine and methylphenidate supports previous findings of commonalities in the neuronal basis of the discriminative effects of these drugs [15]. However, these results stand in stark contrast to the absence of cross-tolerance to the stimulant effect of methylphenidate on locomotor activity.

GENERAL DISCUSSION AND CONCLUSIONS

It is now apparent that tolerance develops to many behavioral effects of caffeine. In this report we considered at some length the development of tolerance to three actions of caffeine in the rat: stimulation of locomotor activity, rate-decreasing effect on food-reinforced operant responding, and discriminative stimulus effects. However, the characteristics of tolerance are not identical for each of these drug actions; rather, two distinct patterns seem to emerge. Tolerance to caffeine-induced stimulation of locomotor activity develops rapidly and can be nearly maximal 24 hr after the start of daily treatment with 35–40 mg/kg in divided doses [9]. Indeed, significant tolerance is evident within hours after the IP administration of a single 10 mg/kg dose of caffeine (Finn and Holtzman, unpublished observation). The tolerance that develops to stimulation of locomotor activity by caffeine is insurmountable. Dose-response curves are displaced downward, flattening as the animals become completely unresponsive to caffeine, even doses 10–30 times higher than doses that increase activity significantly in control animals. Tolerance induced by caffeine to stimulation of locomotor activity shows impressive pharmacologic specificity for methylxanthines. The stimulation of locomotor activity induced by a variety of nonxanthine psychomotor stimulants is undiminished in rats that are entirely unresponsive to the stimulant effect of caffeine and other methylxanthine derivatives. Finally, abrupt termination of chronic daily treatment with an "adequate" dosage of caffeine, e.g., ≥65–70 mg/kg, results in reproducible decreases in spontaneous locomotor activity, suggestive of a withdrawal phenomenon [9,14].

The second pattern of tolerance is seen for effects of caffeine on operant responding and for the discriminative effects of the drug. Unfortunately, temporal and pharmacologic parameters of this type of tolerance have not been characterized as extensively as they have for caffeine-induced stimulation of locomotor activity. Hence, the following points, of necessity, are tentative and subject to change as more information becomes available. Tolerance appears to develop less rapidly to discriminative and operant behavioral effects of caffeine than it does to caffeine-induced stimulation of locomotor activity. Although this temporal variable has not been examined specifically, it is possible to construct stimulus generalization curves for caffeine by cumulative dosing [16]. This is not possible with locomotor activity: substantial tolerance develops to the stimulant effect of caffeine *during* the cumulative dosing procedure (Finn and Holtzman, unpublished observation).

TABLE 3

APPARENT DIFFERENCES IN CHARACTERISTICS OF TOLERANCE TO DIFFERENT BEHAVIORAL EFFECTS OF CAFFEINE IN THE RAT

	Stimulation of Locomotor Activity	Discriminative Effects and Rate-Decreasing Effects on Operant Responding
Rate of development:	rapid	gradual
Type:	insurmountable (complete)	surmountable (incomplete)
Pharmacologic specificity:	xanthines only	nonxanthine psychomotor stimulants
Postwithdrawal changes:	reduced activity	inconsistent

This second type of tolerance is surmountable. Dose-response curves are shifted to the right with no decrement in peak drug effect, even with chronic daily doses of caffeine that are sufficient to induce complete (i.e., insurmountable) tolerance to stimulation of locomotor activity (this report, and [6,16]). The tolerance induced by caffeine extends to nonxanthine compounds, to methylphenidate in the case of discriminative effects. Lastly, postwithdrawal changes in behavior, such as decreases in rate of operant responding, may occur (e.g., [6]), but are not always observed upon termination of chronic daily treatment with doses of caffeine sufficient to result in reduced spontaneous locomotor activity.

The differences in characteristics of these two types of caffeine tolerance are summarized in Table 3. They are derived largely from studies performed on the rat, and to a lesser extent, the mouse, and their applicability to other species remains to be determined.

A final issue is the role of central adenosine systems in the etiology of caffeine tolerance. Caffeine clearly antagonizes behavioral depressant effects of adenosine analogs in rodents, apparently by a competitive interaction at the level of adenosine receptors [19,25]. Furthermore, chronic daily administration of caffeine increases the number of adenosine binding sites in brain. However, the adenosine-antagonist action of caffeine remains in evidence, even in animals completely tolerant to caffeine-induced stimulation of locomotor activity. In those animals, the potency of the adenosine analog, R(−)-PIA, as a behavioral depressant is not increased. Such increased potency would be expected to occur if caffeine tolerance is the consequence of increased functional sensitivity of central adenosine systems, although such apparent increased functional sensitivity has been reported by others [1,27]. Moreover, if the mechanism of caffeine tolerance is an up regulation of adenosine receptors the tolerance should be surmountable. The 25–100% increases in number of adenosine binding sites associated with chronic caffeine administration [1,5] hardly seems adequate to neutralize effects of caffeine doses 10-or-more-fold higher than doses that stimulate locomotor activity in otherwise drug-free control animals.

Tolerance to the discriminative and operant behavioral effects of caffeine has characteristics that are more consis-

tent with increased sensitivity of adenosine systems as an underlying mechanism than does tolerance to caffeine-induced stimulation of locomotor activity (Table 3). Moreover, the sensitivity of adenosine systems during chronic treatment with caffeine usually has been assessed with drugs that are selective for the A_1-adenosine receptor, such as R(−)-PIA [25]. Comparable experiments need to be performed with adenosine analogs that are selective for the A_2-adenosine receptor. It is also possible that the adenosine-antagonist properties of caffeine and the up-regulation of adenosine receptors during chronic caffeine treatment are epiphenomena unrelated to the etiology of tolerance to effects of caffeine on behavior.

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