0022-3565/87/2421-0179$00.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 242, No. 1
Printed in U.S.A.

Psychomotor Stimulant Effects of Caffeine Alone and in Combination with an Adenosine Analog in the Squirrel Monkey

JONATHAN L. KATZ and STEVEN R. GOLDBERG

Preclinical Pharmacology Branch, National Institute on Drug Abuse Addiction Research Center, Baltimore, Maryland and Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland

Accepted for publication April 7, 1987

ABSTRACT

Responding of squirrel monkeys was maintained under a fixed-interval schedule of food presentation in one group of subjects or electric-shock presentation in a second group. Rates of responding were decreased in a dose-dependent manner by the (−)-isomer of ^{6}N-[1-methyl-2-phenylethyl]-adenosine (PIA) under both schedules. Caffeine shifted the (−)-PIA dose-effect curve to the right in a dose-dependent manner. Single administrations of 15.0 mg/kg of caffeine had (−)-PIA-antagonist effects for up to 48 hr. Caffeine administered alone increased rates of responding at intermediate doses, and decreased rates at the highest doses. The increases in response rates produced by intermediate doses of caffeine were appreciably diminished at 24 hr. Doses of (−)-PIA that were inactive when administered alone had little effect on the increases in response rate produced by caffeine. Doses of (−)-PIA that decreased response rates when administered alone attenuated the increase in response rates produced by caffeine. Increases in response rates produced by (+)-amphetamine were altered by (−)-PIA similarly to the manner in which (−)-PIA altered the effects of caffeine. Increases in response rates produced by caffeine were altered by chlorpromazine similarly to the manner in which (−)-PIA altered effects of caffeine. When caffeine was administered daily, as one 15.0-mg/kg injection after experimental sessions, tolerance developed to the response rate increasing effects of caffeine. During the chronic administration of caffeine, the (−)-PIA dose-effect curve was shifted to the right to a degree comparable to the degree that 15.0 mg/kg of caffeine shifted the (−)-PIA dose-effect curve when administered acutely. These results suggest that the psychomotor stimulant effects of caffeine do not occur under all conditions in which caffeine has adenosine-receptor antagonist actions.

Prominent behavioral effects of caffeine in laboratory animals include increased locomotor activity (Dews, 1953; Snyder *et al.*, 1981) and increases in the frequency of occurrence of learned behaviors (Davis *et al.*, 1973; Skinner and Heron, 1937). These effects, along with convulsions at high doses, are the distinguishing features of psychomotor stimulant drugs (Kelleher, 1977). Recent studies have suggested that the psychomotor stimulant effects of caffeine are due to an antagonism of the effects of endogenous adenosine at adenosine receptors in brain (Snyder *et al.*, 1981; Katims *et al.*, 1983). These suggestions have been made inasmuch as the brain concentrations at which methylxanthines have psychomotor stimulant effects are similar to those at which the drugs have antagonist actions at adenosine receptors. Additionally, there is a correlation in potencies of a variety of methylxanthines for increasing locomotor activity and for displacing [^{3}H]cyclohexyladenosine from A_1-adenosine receptors (Snyder *et al.*, 1981; Katims *et al.*, 1983).

Several behavioral studies have confirmed that, as *in vitro* (Sattin and Rall, 1970), caffeine can antagonize effects of adenosine and its analogs (Coffin and Carney, 1983; Glowa and Spealman, 1984; Snyder *et al.*, 1981; Sirochman and Carney, 1981). However, if effects of caffeine alone are due to its antagonist actions at adenosine receptors, then they should be attenuated when adenosine agonists are administered with caffeine (Glowa and Spealman, 1984; Goldberg *et al.*, 1985). A few studies suggest that effects of caffeine are not altered by adenosine receptor agonists. For example, rats trained in caffeine-discrimination procedures to emit one response after administration of caffeine and an alternate response after administration of vehicle, show increased levels of drug-appropriate responding with increasing caffeine dose. The adenosine analog (−)-PIA failed to antagonize the discriminative effects of caffeine (Holloway *et al.*, 1985). In another study, alterations in temporal patterns of responding in rats under fixed-interval schedules produced by caffeine were not antagonized by (−)-PIA (Goldberg *et al.*, 1985).

The present study examined further the effects of interactions of caffeine and (−)-PIA on responding maintained under fixed-interval schedules. Of specific interest was the increase in response rates under fixed-interval schedules produced by

Received for publication June 27, 1986.

ABBREVIATION: PIA, ^{6}N-[1-methyl-2-phenylethyl]-adenosine.

caffeine. Thus, the present study examined the increases in response rates produced by caffeine when administered alone and when administered with (−)-PIA. Because when administered alone (−)-PIA decreases response rates, the alteration of the effects of caffeine by (−)-PIA might be due to the addition of these opposing effects, a physiological antagonism. Therefore, the present study compared the effects of the interactions of caffeine and (−)-PIA with the interactions of (+)-amphetamine and (−)-PIA as well as the interactions of chlorpromazine and caffeine. If the effects of combinations of caffeine and (−)-PIA were a result of a physiological antagonism, then the three interactions should not be appreciably different. Finally, studies were conducted of the effects of caffeine and (−)-PIA in subjects rendered tolerant to the response-rate increasing effects of caffeine in order to determine if tolerance also develops to the adenosine-antagonist effects of caffeine.

Methods

Subjects. Nine adult male squirrel monkeys (*Saimiri Sciureus*) were studied. For those studied under schedules of food reinforcement, the daily food ration (Purina Monkey Chow supplemented with Teklad Monkey Diet) was adjusted to maintain their body weights at 80 to 85% of those maintained during unrestricted feeding. Water was always available in the individual home cages. All monkeys had been studied previously under experimental procedures similar to those described below and had received injections of morphine, ethylketazocine and naloxone no more frequently than once per week. In the initial stages of the present study, caffeine and (−)-PIA effects were studied once per week with opioids studied once per week; at least 3 days separated injections (Katz and Goldberg, 1986). Subjects used for determinations of effects of each drug alone or drug combinations under schedules of food or electric-shock presentation are shown in table 1.

Apparatus. During experimental sessions, subjects were seated and restrained loosely about the waist in Plexiglas chairs (Barrett, 1985; Hake and Azrin, 1963) which were placed within ventilated, sound-attenuating chambers (model AC-3, Industrial Acoustics Co., Bronx, NY) that were provided with continuous white noise to mask extraneous sounds. Mounted on the front panel of each chair was a response key (model 121-05, BRS/LVE, Laurel, MD) on which a downward force of at least 20 g produced an audible click and was recorded as a response. Mounted behind the clear front panel of the chair where three pairs of stimulus lamps (7 w, 120 V a.c.) which were colored differently and could be illuminated individually. A food-pellet dispenser (model D-1, Ralph Gerbrands Co. Arlington, MA) could deliver 190-mg food pellets (banana flavored, BioServ Inc., Frenchtown, NJ) to a tray accessible through an opening in the front panel of the chair. Below the seat of the chair was a stock which restrained the tail of the monkey for presentation of electric shock (200-msec pulse, 5 mA, 650 V a.c., 60 Hz). Shock was delivered through a brass electrode which rested on a shaved portion of the tail. Relatively low resistance contact was ensured by coating the tail with electrode paste (EKG Sol, Burton, Parsons and Co., Washington, DC) and by delivering the shock through a high in-series resistance.

TABLE 1

Subjects studied with each drug and drug combination under the fixed-interval schedules of food or electric-shock presentation

Drug Treatments	Food Presentation	Shock Presentation
(−)-PIA alone and with caffeine	S-52, S-266, S-980	S-181, S-280, S-580, S-880
Caffeine alone and with (−)-PIA[a]	S-52, S-266, S-980	S-181, S-280, S-580, S-880
Caffeine with daily caffeine	S-266, S-881, S-980	S-181, S-580, S-880
(−)-PIA with daily caffeine	S-266, S-881, S-980	S-181, S-580, S-880
(−)-PIA with daily caffeine and omissions	S-266, S-881, S-980	S-181, S-880
(−)-PIA with caffeine, 24 to 72 hr prior	S-52, S-881	S-181, S-280, S-880
(+)-Amphetamine alone and with (−)-PIA	S-52, S-54, S-881	S-181, S-280, S-880
Caffeine with chlorpromazine	S-52, S-54, S-881	S-181, S-280, S-880

[a] Caffeine with (−)-PIA doses: 0.04: S-52, S-266, S-980, S-181, S-580, S-880; 0.39: S-52, S-266, S-980, S-181, S-280, S-880; and 1.16: S-52, S-266, S-980, S-181, S-880.

Behavioral procedures. Key-press responding was maintained under fixed-interval schedules of either food presentation or electric-shock presentation during experimental sessions that were conducted daily, 5 days/week. For monkeys responding under the schedule of food presentation, the green stimulus lamps were illuminated and the first response after the lapse of 180 sec produced a food pellet accompanied by the extinguishing of the green lamps and a 200-msec flash of white stimulus lamps. A 60-sec timeout period followed each food presentation during which all stimulus lamps were out and responding had no scheduled consequences. Sessions ended after the 10th timeout period. For monkeys responding under the schedule of electric-shock presentation, the red stimulus lamps were illiminated and the first response after 180 sec produced electric shock accompanied by the extinguishing of the red lamps and a 200-msec flash of white stimulus lamps. Each shock was followed by a 1-min timeout and sessions ended after the 10th timeout period.

Subjects studied under the schedule of electric-shock presentation were trained initially to respond under schedules of shock postponement; when responding was well maintained (when few electric shocks were presented) the schedule was changed to one in which responses postponed shock and, in addition, the first response after 180 sec produced shock. Subsequently, the shock postponement schedule was removed and the sole consequence of responding was electric-shock presentation. Several sessions later the timeout was added. This procedure follows that described by McKearney (1968).

Drugs and injection procedures. The base form of (−)-PIA (Boehringer Mannheim Corp., New York, NY) was dissolved in 0.1 N HCl and diluted with saline (0.9% NaCl) to achieve the appropriate concentration. The (−)-isomer of PIA shows the rectus absolute configuration. Caffeine sodium benzoate (Sigma Chemical Co., St. Louis, MO), (+)-amphetamine sulfate (Arenol) and chlorpromazine hydrochloride (Sigma) were dissolved in saline. Doses were injected i.m. (calf or thigh) in a volume of 1.0 ml/kg b.wt. or less. Control injections were similar volumes of saline. Drugs were injected i.m. (calf or thigh) 5 min before experimental sessions. When two drugs were administered, each was injected in a different leg. All doses are expressed as milligrams of the base per kilogram of the body weight of the subject.

Effects of drugs administered before experimental sessions were, throughout the study, assessed no more frequently than twice per week, typically Tuesdays and Fridays. Either a noninjection- or vehicle-control session, with characteristic rates and temporal patterns of responding, preceded each session in which drug effects were assessed. Vehicle-control sessions were conducted each Thursday and data from these sessions served as the control reference. Doses of each drug or drug combination were studied in a mixed sequence with a complete dose-effect curve determined before another drug or drug combination was studied. Doses of each drug or drug combination were studied once or twice in each subject.

In some studies of effects of the two drugs in combination, caffeine was administered 24, 48, or 72 hr before (−)-PIA. In these studies experimental procedures were conducted as usual during the interim between doses of caffeine and (−)-PIA.

In studies of chronic caffeine administration, doses of caffeine were administered 7 days/week; on days on which experimental sessions were conducted, caffeine was administered 2 to 3 hr after experimental

sessions. For three monkeys (S-52, S-266 and S-980) studied under the schedule of food presentation, the dose was initially 28.0 mg/kg. After 3 days at this dose one monkey (S-52) had stopped responding and was losing weight. The dose was lowered to 15.0 mg/kg on the 4th day, however, S-52 did not regain weight or resume responding within 2 days. Therefore, caffeine administration was stopped for S-52 and S-881 was used instead. The replacement subject, and all subjects studied under the schedule of shock presentation, were given 15.0 mg/kg of caffeine from the start of chronic administration. When effects of (−)-PIA or caffeine on responding were studied during the chronic caffeine regimen, doses of either drug were administered i.m. 5 min before experimental sessions. When effects of caffeine doses lower than 15.0 mg/kg were administered before sessions, the remainder of the daily caffeine dose was administered after the sessions.

In some studies of effects of (−)-PIA during the chronic caffeine regimen, the usual dose of caffeine was omitted on 1 or 2 days preceding experimental sessions in which effects of (−)-PIA were assessed. Caffeine administration was resumed in the afternoon of the day in which (−)-PIA effects were assessed. Single-day omissions of caffeine were studied no more frequently than twice per week. Two-day caffeine omissions were studied no more frequently than once per week.

Measurement of effects. Overall rates of responding were computed each session by dividing total responses by elapsed time for individual subjects. Effects of drugs in individual subjects were considered significant when overall rate of responding after drug differed from the average control response rate of that subject by at least ± 2 S.D.s. Response rates at each drug or drug combination are shown in the figures as the mean of percentages of vehicle-control sessions for all subjects, and are referred to in the text as average response rates. For comparisons of some dose-effect curves, analysis of variance and linear regression techniques (Snedecor and Cochran, 1967) were used to determine ED_{50} values (the dose causing a decrease in response rates to 50% of the control) and the 95% CL.

Results

Control performances. Under the fixed-interval schedules of food or electric-shock presentation, performances were similar to those observed previously (Ferster and Skinner, 1957; McKearney, 1968). At the start of the interval there was a pause followed by increasing response rates up to food or shock presentation (figs. 1 and 2; Control). Generally, pauses were shorter and response rates higher under the schedule of electric-shock presentation.

Effects of caffeine alone and in combination with (−)-PIA. Caffeine when administered alone increased average rates of responding under the fixed-interval schedules (fig. 3, filled symbols). Significant increases in response rates occurred in all subjects at doses of 1.5 and 5.0 mg/kg under the schedules of food and shock presentation, respectively. A dose of 28.0 mg/kg decreased response rates significantly in all subjects under the schedule of food presentation. A dose of 50.0 mg/kg decreased response rates significantly in two of four subjects under the schedule of shock presentation.

Under the schedule of food presentation, a low dose of (−)-PIA (0.04 mg/kg) that by itself did not alter response rates significantly in any subjects, slightly attenuated the increases in average response rates produced by caffeine (fig. 3, left panel; compare ● with △). Significant increases were obtained in only one of the three subjects studied with a combination of 0.04 mg/kg of (−)-PIA and 1.5 mg/kg of caffeine. In combination with doses of (−)-PIA (0.39 and 1.16 mg/kg) that decreased response rates when administered alone, caffeine did not increase average rates of food-maintained responding. Only one of three subjects showed a significant increase at 0.39 mg/kg of

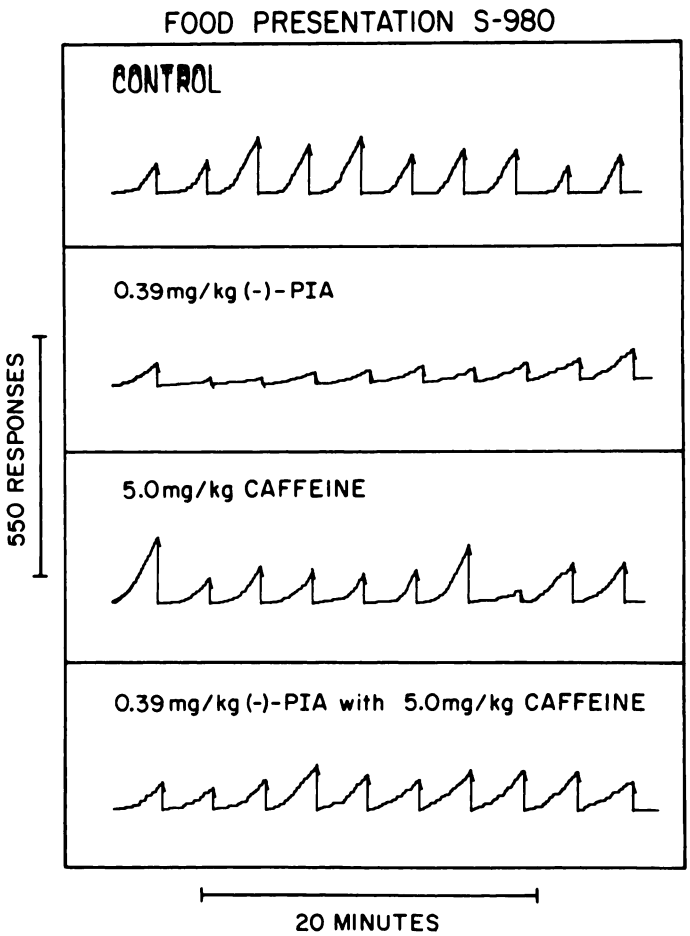

Fig. 1. Representative performances of squirrel monkey S-980 under the fixed-interval 3-min schedule of food presentation. Abscissae: time; ordinates: cumulative responses. Slash marks represent presentations of food. The cumulative curve resets to base at the beginning of each fixed interval. Note that the decreases in response rates produced by 0.39 mg/kg of (−)-PIA were antagonized by 5.0 mg/kg of caffeine. Note also that the temporal patterns of responding after the combination of drugs are not appreciably different from those obtained after vehicle injections.

(−)-PIA and 1.5 mg/kg of caffeine. All of the subjects showed significant decreases in response rates after combinations of 1.16 mg/kg of (−)-PIA and 1.5 mg/kg of caffeine. Caffeine at low to intermediate doses attenuated the decreases in average response rates produced by (−)-PIA in a dose-related manner (fig. 3; ◇ and □). Decreases in average response rates produced by the highest dose of caffeine were not affected systematically by the different (−)-PIA doses that were coadministered.

Under the schedule of shock presentation, a low dose of (−)-PIA (0.04 mg/kg) that by itself did not alter response rates significantly in any subjects, did not attenuate the increases in average rates of responding produced by caffeine (fig. 3; right panel; compare ● with △). Significant increases were obtained in all subjects at 5.0 mg/kg of caffeine, the dose that produced significant rate increases in all subjects when administered alone. The 0.39-mg/kg dose of (−)-PIA, dose that decreased average response rates when administered alone, also was generally ineffective in eliminating the increases in average rates of shock-maintained responding produced by caffeine (fig. 3; right panel; compare ● and ◇). At this dose combination, significant increases in response rates were obtained in all subjects at the 15.0-mg/kg dose of caffeine. Decreases in aver-

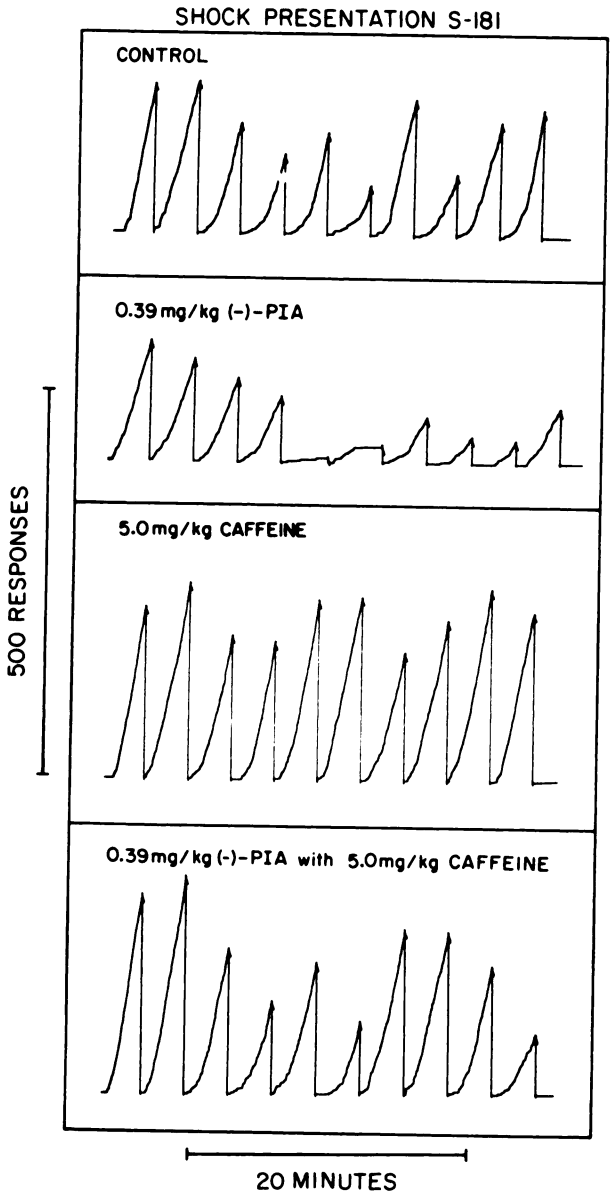

Fig. 2. Representative performances of squirrel monkey S-181 under the fixed-interval 3-min schedule of electric-shock presentation. Abscissae: time; ordinates: cumulative responses. Slash marks represent presentations of shock. The cumulative curve resets to base at the beginning of each fixed interval. Note that the decreases in response rates produced by 0.39 mg/kg of (−)-PIA were antagonized by 5.0 mg/kg of caffeine. Note also that the increases in rates of responding after the combination of drugs were not appreciably different from those obtained after injection of 5.0 mg/kg of caffeine alone.

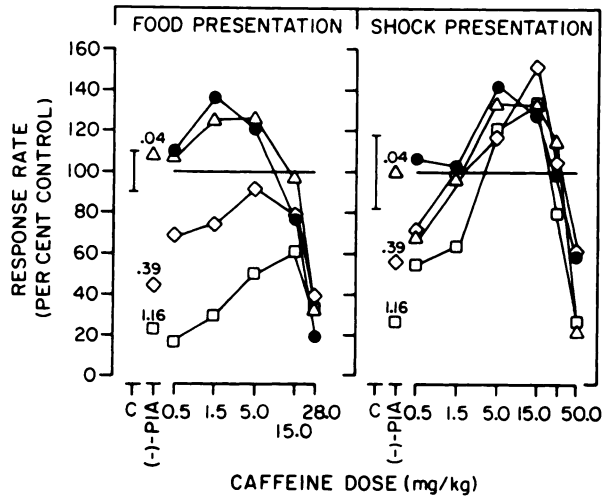

Fig. 3. Effects of caffeine alone and in combination with (−)-PIA on average rates of responding in squirrel monkeys under the fixed-interval 3-min schedules of food or electric-shock presentation. Ordinates: average response rate expressed as a percentage of control (C) response rates; abscissae: dose of caffeine in milligrams per kilogram, log scale. Vertical bars above C show ± 1 S.D. of C values. ●, effects of caffeine administered alone. The unconnected open points above (−)-PIA show effects of doses of (−)-PIA administered alone; open connected points show effects of caffeine given in combination with corresponding doses of (−)-PIA. Note that caffeine increased response rates and that the rate-increasing effects of caffeine were only attenuated by doses (−)-PIA that, when given alone, decreased response rates.

age response rates produced by the highest dose of caffeine were either not affected systematically or decreased further by the coadministration of (−)-PIA.

Figure 2 shows decreases in rates of shock-maintained responding produced by 0.39 mg/kg of (−)-PIA, effects of 5.0 mg/kg of caffeine and the effects of the combination of those doses. The increases in rates of responding produced by caffeine were not appreciably altered by the coadministration of (−)-PIA. Furthermore, the disruption in the temporal patterns of responding obtained with caffeine alone were also obtained when the two drugs were given in combination. Compared to control performances, after both caffeine alone and combinations of caffeine and (−)-PIA, responding was initiated earlier in the interval. Decreases in response rates produced by 50.0 mg/kg of caffeine were either not affected or greater when (−)-PIA was also administered.

The duration of effects of caffeine on overall rates of responding under the schedule of shock presentation are shown in figure 4. When administered 5 min before the session, caffeine increased response rates significantly in all subjects at 5.0 mg/kg and in two of three subjects at 15.0 mg/kg. When caffeine was administered 24 and 48 hr before the session, the increases in average response rates were not as great (fig. 4, ○ and ◇) and were only significant in one subject at 15.0 mg/kg).

Effects of (−)-PIA alone and in combination with caffeine. Average rates of responding were decreased in a dose-related manner by (−)-PIA (fig. 5; filled symbols). A dose of 0.39 mg/kg decreased response rates significantly under the schedules of food or shock presentation in all subjects. Under the schedule of food presentation, a dose of 5.0 mg/kg of caffeine shifted the (−)-PIA dose about one-half log U to the right (fig. 5, left panel). The ED_{50} value for (−)-PIA alone was 0.41 mg/kg (95% CL: 0.35–0.48 mg/kg); with 5.0 mg/kg of caffeine the ED_{50} value for (−)-PIA was 1.21 mg/kg (95% CL: 0.77–1.90 mg/kg). Figure 1 shows decreases in rates of food-maintained responding produced by 0.39 mg/kg of (−)-PIA, effects of 5.0 mg/kg of caffeine and the effects of the combination of those doses. The decreases in rates of responding produced by (−)-PIA were restored to near control levels by coadministration of caffeine. A higher dose of caffeine (15.0 mg/kg) also shifted the (−)-PIA dose-effect curve to the right (fig. 5; left panel). This dose of caffeine decreased response rates when administered alone. The ED_{50} values for the parallel portion of this curve was 1.71 mg/kg (95% CL: 0.71–4.12).

Under the schedule of shock presentation, a dose of 0.5 mg/kg of caffeine shifted the (−)-PIA dose-effect curve less than one-half log U to the right (fig. 5; right panel). The ED_{50} values

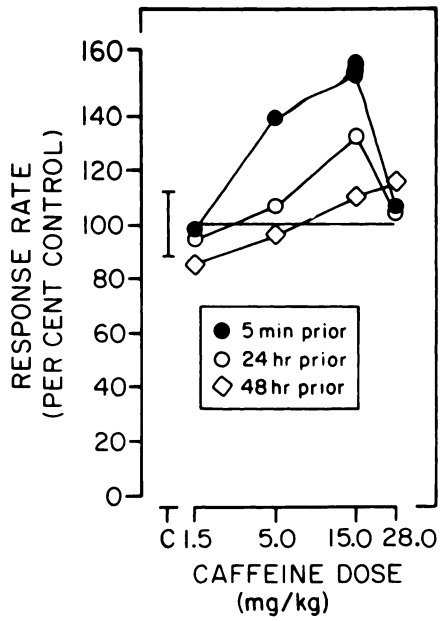

Fig. 4. Effects of caffeine on average rates of responding in squirrel monkeys under the fixed-interval 3-min schedule of electric-shock presentation shown at different times after administration. Ordinates: average response rate expressed as a percentage of control (C) response rates; abscissae: dose of caffeine in milligrams per kilogram, log scale. The vertical bar above C shows ± 1 S.D. of C values. ●, effects of caffeine administered 5 min before sessions; ○, effects of caffeine given 24 hr before the session; ◇, effects of caffeine given 48 hr before sessions. Note that the increase in response rates produced by caffeine were attenuated by 24 hr after injection and were eliminated 48 hr after injection.

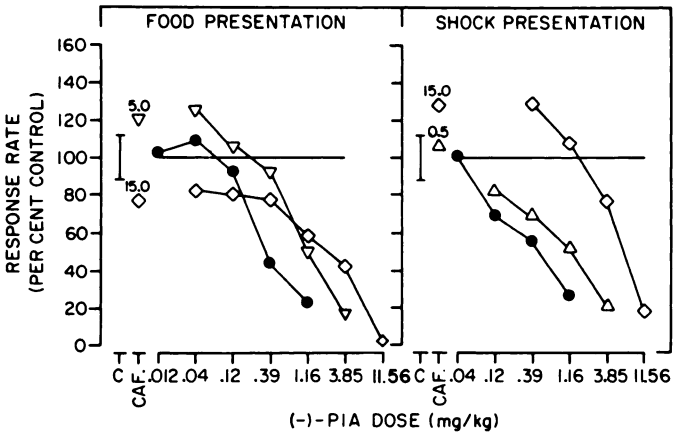

Fig. 5. Effects of (−)-PIA alone and in combination with caffeine (CAF) on average rates of responding in squirrel monkeys under the fixed-interval 3-min schedules of food or electric-shock presentation. Ordinates: average response rates expressed as a percentage of control (C) response rates; abscissae: dose of (−)-PIA in milligrams per kilogram, log scale. Vertical bars above C show ± 1 S.D. of C values. ●, effects of (−)-PIA administered alone. The unconnected open points above CAF show effects of doses of CAF administered alone; open connected points show effects of (−)-PIA given in combination with corresponding doses of CAF. Note that CAF shifted the (−)-PIA dose effect curve to the right of a degree that depended on the dose of CAF.

for (−)-PIA alone (0.41 mg/kg; 95% CL: 0.17–0.98 mg/kg) and with 0.5 mg/kg of caffeine (0.73 mg/kg; 95% CL: 0.39–1.38 mg/kg) were not significantly different. A higher dose of caffeine (15.0 mg/kg), which increased response rates when administered alone, shifted the (−)-PIA dose-effect curve to the right by about one log U (fig. 5; right panel). The ED_{50} value for (−)-PIA with 15.0 mg/kg of caffeine was 6.30 mg/kg (95% CL: 3.07–12.94 mg/kg).

Under either condition, 15.0 mg/kg of caffeine antagonized the decreases in average response rates produced by 1.16 mg/kg of (−)-PIA maximally when the two drugs were given in combination 5 min before the session and to a lesser extent when caffeine was given at increasing lengths of time before (−)-PIA (fig. 6). Significant decreases in all subjects under both conditions were obtained at 1.16 mg/kg of (−)-PIA given alone. When both (−)-PIA and caffeine were administered under the schedule of food presentation, significant decreases in all subjects were observed only when caffeine was given 48 hr before (−)-PIA. Under the schedule of shock presentation, significant decreases with combinations of 1.16 mg/kg of (−)-PIA and 15.0 mg/kg of caffeine were only obtained in one subject when caffeine was given 72 hr before the (−)-PIA.

Effects of (−)-PIA in combination with (+)-amphetamine. Under the schedule of food presentation, (+)-amphetamine increased average response rates at an intermediate dose (0.04 mg/kg); higher doses decreased average response rates (fig. 7; left panel; ●). Increases in response rates were significant in all subjects at 0.04 mg/kg. A low, ineffective dose (0.04 mg/kg) of (−)-PIA attenuated the increases in average response rates (fig. 7: left panel; compare ● and △); increases in response rates at 0.04 mg/kg of (+)-amphetamine with (−)-PIA were only significant in one of the three subjects. Significant increases in response rates were not obtained in any subjects at

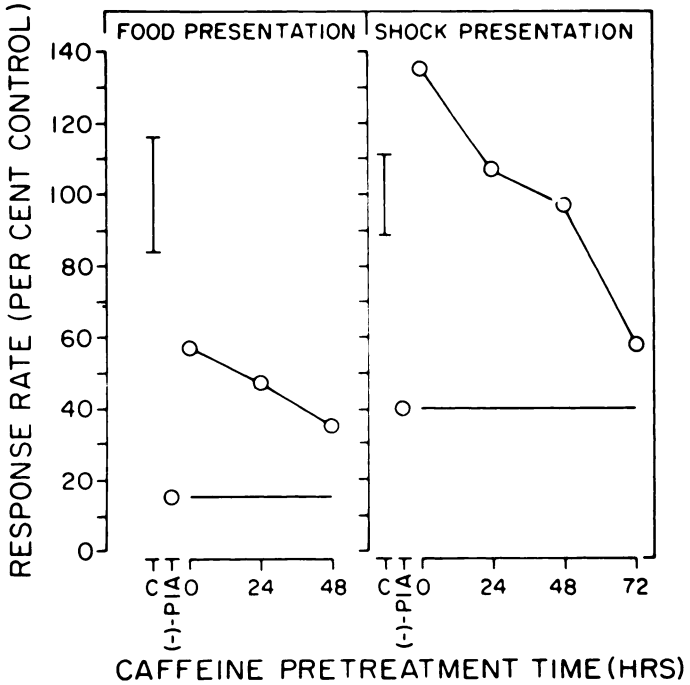

Fig. 6. Effects of caffeine on the decreases in response rates produced by 1.16 mg/kg of (−)-PIA as a function of the time since caffeine administration. Ordinates: average response rates expressed as a percentage of control (C) response rates: abscissae: caffeine pretreatment time in hours. Vertical bars above C show ± 1 S.D. of C values. The unconnected circle shows effects of (−)-PIA given alone, 5 min before the session. The connected points show effects of 15.0 mg/kg of caffeine given at the respective number of hours before a session that followed by 5 min a (−)-PIA injection. Note that the antagonist effect of caffeine was greatest when given at the same time as (−)-PIA under the schedule of food presentation and diminished as a function of time since the injection. Under the schedule of shock presentation, caffeine antagonized completely the effects of (−)-PIA for up to 48 hr after its injection.

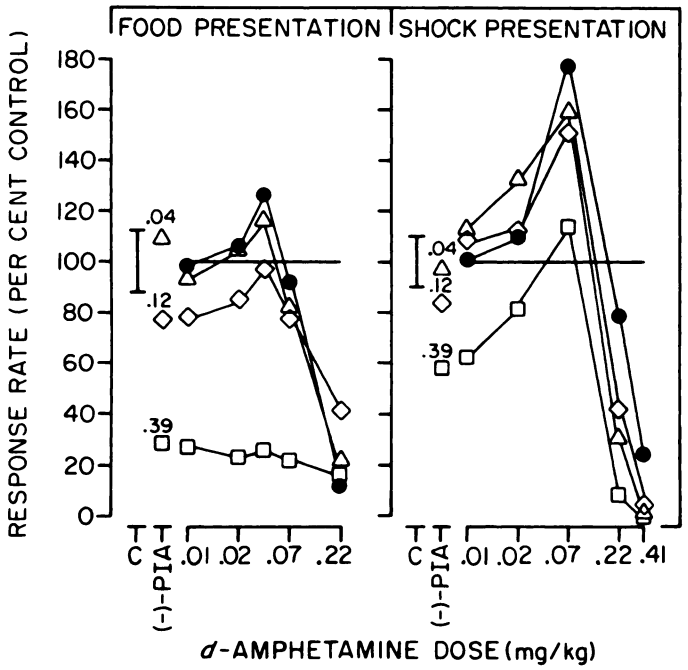

Fig. 7. Effects of (+)-amphetamine alone and in combination with (−)-PIA on average rates of responding in squirrel monkeys under the fixed-interval 3-min schedules of food or electric-shock presentation. Ordinates: average response rates expressed as a percentage of control (C) response rates; abscissae: dose of (+)-amphetamine in milligrams per kilogram, log scale. Vertical bars above C show ± 1 S.D. of C values. ●, effects of (+)-amphetamine administered alone. The unconnected open points above (−)-PIA show effects of doses of (−)-PIA administered alone; open connected points show effects of (−)-PIA given in combination with corresponding doses of (+)-amphetamine. Note that the increases in response rates produced by (+)-amphetamine were most attenuated by doses of (−)-PIA that decreased response rates when administered alone.

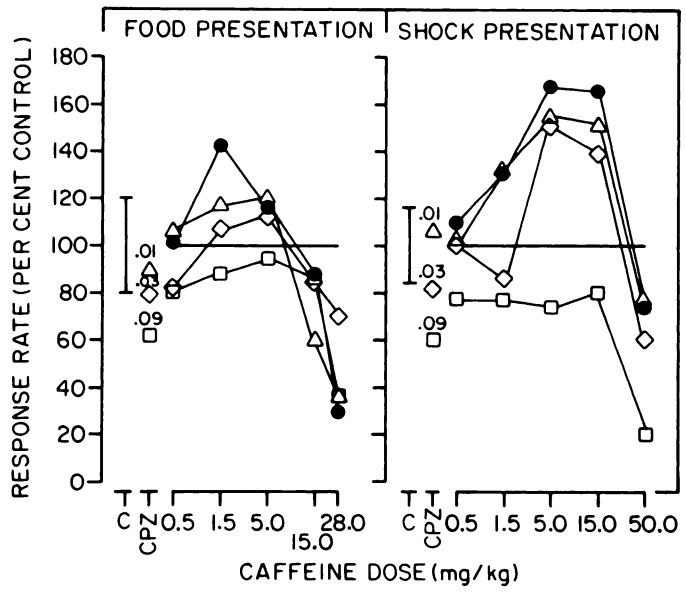

Fig. 8. Effects of caffeine alone and in combination with chlorpromazine (CPZ) on average rates of responding in squirrel monkeys under the fixed-interval 3-min schedules of food or electric-shock presentation. Ordinates: average response rate expressed as a percentage of control (C) response rates; abscissae: dose of caffeine in milligrams per kilogram, log scale. Vertical bars above C show ± 1 S.D. of C values. ●, effects of caffeine administered alone. The unconnected open points above CPZ show effects of doses of CPZ administered alone; open connected points show effects of caffeine given in combination with corresponding doses of CPZ. Note that caffeine increased response rates and that the rate-increasing effects of caffeine under the schedule of food presentation were attenuated by doses of CPZ that, when given alone, were inactive or decreased response rates. Under the schedule of electric-shock presentation, increases in response rates produced by caffeine were most attenuated by doses of CPZ that, when given alone, decreased response rates.

any doses of (+)-amphetamine given in combination with higher doses of (−)-PIA (0.39 and 1.16 mg/kg) that, when given alone, decreased response rates (fig. 7; left panel; ◇ and □).

Average rates of responding under the schedule of shock presentation were increased by an intermediate dose of (+)-amphetamine (0.07 mg/kg) and the increase was greater than that under the schedule of food presentation. Higher doses decreased average response rates (fig. 7; right panel; ●). Increases in response rates were significant in all subjects at 0.07 mg/kg. Low doses of (−)-PIA, that alone either did not affect or decreased average response rates (0.04 and 0.12 mg/kg, respectively), attenuated the rate-increasing effects of (+)-amphetamine (fig. 7; right panel; compare circles and triangles or diamonds). Increases in response rates were significant in two of three subjects at 0.07 mg/kg of (+)-amphetamine with 0.04 mg/kg of (−)-PIA, and in only one subject at the higher doses of (−)-PIA. Decreases in average response rates at high doses of (+)-amphetamine were enhanced slightly by the coadministration of (−)-PIA. At low to intermediate doses of (+)-amphetamine, the decreases in average response rates produced by (−)-PIA were reversed in a dose-dependent manner by (+)-amphetamine.

Effects of chlorpromazine in combination with caffeine. Increases in average rates of food-maintained responding produced by caffeine were attenuated by chlorpromazine administration (fig. 8; left panel). For example, 1.5 mg/kg of caffeine administered alone increased response rates significantly in two of three monkeys but did not increase response rates significantly in any monkeys when administered in combination with any dose of chlorpromazine. Chlorpromazine was less effective in attenuating the increases in average response rates produced by caffeine under the schedule of shock presentation. Doses of 0.01 and 0.03 mg/kg only slightly attenuated the increases in average response rates produced by caffeine (fig. 8; right panel; compare △ and ◇ to ●). Caffeine administered alone at doses of 5.0 or 15.0 mg/kg, increased response rates significantly in three or two of three subjects, respectively. When administered with 0.01 mg/kg of chlorpromazine, significant increases in response rates were also obtained at those doses in those subjects. At a chlorpromazine dose of 0.03 mg/kg, significant increases in response rates were again obtained at those doses in the same number of subjects. At a chlorpromazine dose of 0.03 mg/kg, significant increases in response rates were obtained in all subjects at 5.0 mg/kg of caffeine and in one subject at 15.0 mg/kg. The increases in response rates produced by caffeine were eliminated by 0.1 mg/kg of chlorpromazine, a dose that decreased response rates when administered alone (fig. 8; right panel; squares).

Effects of chronic caffeine administration. Daily administration of caffeine after experimental sessions generally did not alter the control response rates (table 2). Effects of caffeine on average response rates given before sessions during the repeated administration of caffeine are shown in figure 9. In contrast to effects obtained before chronic administration, under either the schedule of food or electric-shock presentation, low to intermediate doses (0.5 to 5.0 mg/kg) did not increase

TABLE 2

Average of control response rates (±1 S.D.) during the determinations of dose-effect curves before and during chronic caffeine administration

Subject	Condition	Before Chronic Caffeine	During Chronic Caffeine
S-181	Shock	0.76	0.63
		(0.14)	(0.10)
S-280	Shock	1.31	
		(0.22)	
S-580	Shock	1.06	1.10
		(0.09)	(0.12)
S-880	Shock	1.48	1.49
		(0.26)	(0.22)
S-52	Food	0.37	
		(0.05)	
S-54	Food	0.29	
		(0.05)	
S-266	Food	0.27	0.26
		(0.03)	(0.03)
S-881	Food	0.66	0.44
		(0.09)	(0.06)
S-980	Food	0.54	0.45
		(0.07)	(0.08)

average response rates. Higher doses generally decreased response rates; under the schedule of shock presentation, the highest dose decreased average rates to a greater extent than before chronic caffeine administration (fig. 9; open symbols). A significant increase in response rates was obtained in only one subject at only one dose (1.5 mg/kg) during chronic caffeine administration.

During the chronic administration of caffeine, (−)-PIA decreased average rates of responding maintained by either food or electric-shock presentation (fig. 10, ○). The decreases in response rates required higher doses than were necessary to decrease response rates before chronic caffeine administration; dose-effect curves for (−)-PIA were shifted to the right by 0.5 to 1 log Unit. (●, fig. 10, show effects of (−)-PIA before chronic caffeine.) Furthermore, the ED_{50} values were increased significantly from 0.41 mg/kg under either schedule to 1.98 or 3.17 mg/kg under the schedules of food or shock presentation, respectively (table 3). The degree of shift in the (−)-PIA dose-effect curve approximated the degree to which 15.0 mg/kg of caffeine shifted the (−)-PIA dose-effect curve before chronic caffeine administration. (◇, fig. 10, show effects of 15.0 mg/kg of caffeine in combination with (−)-PIA before chronic caffeine administration). In combination with 15.0 mg/kg of caffeine before chronic administration, the ED_{50} values for (−)-PIA were 1.71 mg/kg (95% CL: 0.71–4.12 mg/kg) and 6.30 mg/kg (95% CL: 3.07–12.94 mg/kg) under the food and shock schedules, respectively, and not significantly different from those obtained during the chronic administration of caffeine (table 3).

During the chronic caffeine regimen, a 1- or 2-day omission of caffeine had no appreciable effects on response rates on the following days (data not shown). Administration of (−)-PIA before sessions after caffeine omissions decreased response rates at doses that were comparable to those that decreased rates when caffeine injections were not omitted. For example, under the schedule of food presentation the ED_{50} value for (−)-PIA was 1.98 mg/kg when daily caffeine was not omitted and 1.27 and 1.45 mg/kg when caffeine injections were omitted for 1 or 2 days, respectively. Similar results were obtained under the schedule of electric-shock presentation (see table 3).

Discussion

In the present study caffeine increased rates of responding maintained in squirrel monkeys under fixed-interval schedules of either food or electric-shock presentation. Caffeine also antagonized the effects of the relatively selective A_1-adenosine receptor agonist, (−)-PIA. A number of reports have suggested that the psychomotor-stimulant effects of methylxanthines such as caffeine are due to their adenosine antagonist actions. For example, the relative potencies of methylxanthines for producing increases in locomotor activity were reported to be well correlated with the relative potencies of the drugs for displacement of [^{3}H]cyclohexyladenosine from A_1-adenosine receptors (Snyder *et al.*, 1981). Furthermore, several others have reported that the behavioral effects of adenosine analogs, like *in vitro* effects (Sattin and Rall, 1970), can be antagonized by methylxanthines (*e.g.* Sirochman and Carney, 1981; Coffin *et al.*, 1984; Glowa and Spealman, 1984; Goldberg *et al.*, 1985, 1986; Logan and Carney, 1984).

As noted previously, if the psychomotor stimulant effects of

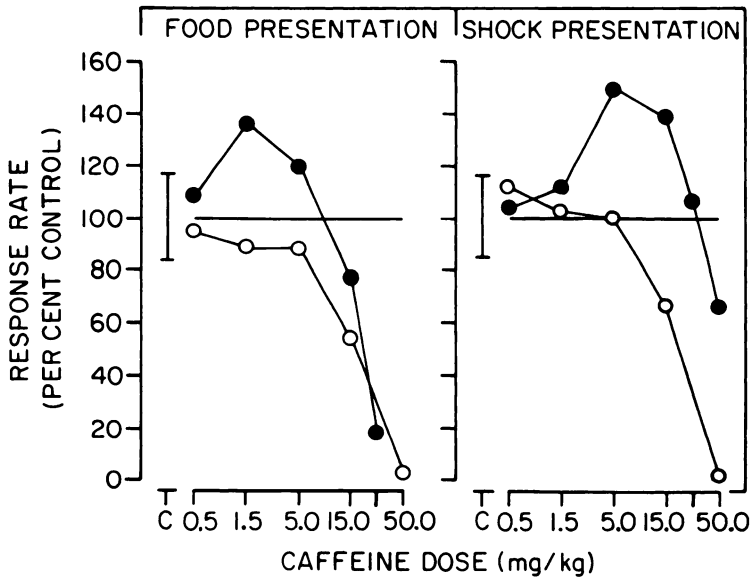

Fig. 9. Effects of caffeine before and during the daily administrations of caffeine on average rates of responding in squirrel monkeys under the fixed-interval 3-min schedules of food or electric-shock presentation. Ordinates: average response rate expressed as a percentage of control (C) response rates; abscissae: dose of caffeine in milligrams per kilogram, log scale. Vertical bars above C show ± 1 S.D. of C values. ●, effects of caffeine administered before the daily administrations of caffeine; effects of caffeine given during the daily administrations of caffeine. Note that caffeine increased response rates before it was administered daily, and that the rate-increasing effects of caffeine were not observed when the drug was administered daily.

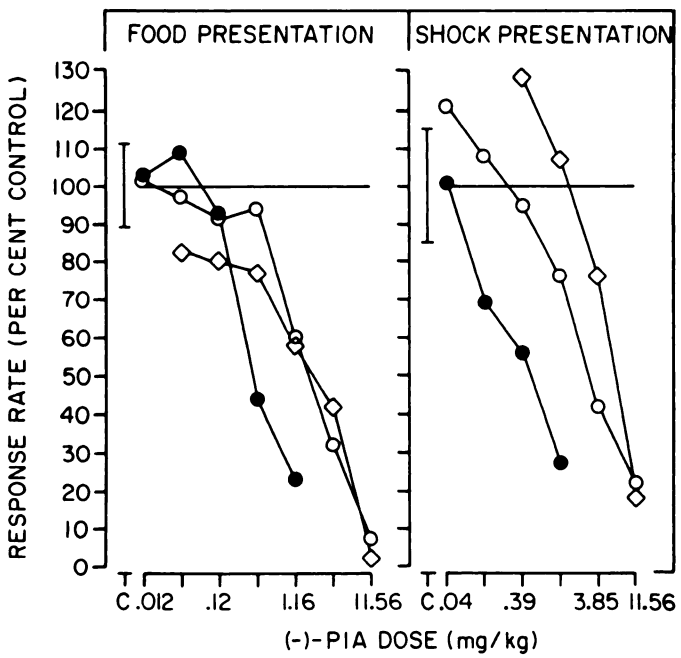

Fig. 10. Effects of (−)-PIA before and during the daily administrations of caffeine on average rates of responding in squirrel monkeys under the fixed-interval 3-min schedules of food or electric-shock presentation. Ordinates: average response rate expressed as a percentage of control (C) response rates; abscissae: dose of (−)-PIA in milligrams per kilogram, log scale. Vertical bars above C show ± 1 S.D. of C values. ●, effects of (−)-PIA administered before the daily administrations of caffeine; ○, effects of (−)-PIA given during the daily administrations of caffeine. ◇, effects of combinations of 30.0 mg/kg of caffeine with (−)-PIA before daily administration of caffeine. Note that during daily administration of caffeine the (−)-PIA dose-effect curve was shifted to the right by about 1 log U and that the shift was comparable to the shift produced by combinations of (−)-PIA and caffeine before the daily administration of caffeine.

TABLE 3

ED_{50} values (with 95% CL) for decreases in response rates produced by (−)-PIA before and during the chronic administration of 15.0 mg/kg of caffeine

The effects of (−)-PIA were determined before and during chronic administration of caffeine after a day with a routine caffeine injection (0d) and after one-day (1d) or two-day (2d) omissions of caffeine.

Event Maintaining Behavior	Before Chronic Caffeine	During Chronic Caffeine		
		0d	1d	2d
Food	0.41 (0.35–0.48)	1.98 (1.30–3.00)	1.27 (0.82–1.96)	1.45 (0.43–4.95)
Shock	0.41 (0.17–0.98)	3.17 (1.33–7.55)	2.40 (0.68–8.40)	2.24* (0.05–96.27)

* Linear regression was not significant.

methylxanthines are due to antagonist actions at adenosine receptors, then the stimulant effects should be diminished when an adenosine-receptor agonist is also administered (Glowa and Spealman, 1984; Goldberg *et al.*, 1985). In the present study the increases in response rates under fixed-interval schedules produced by caffeine were attenuated by the coadministration of selected doses of (−)-PIA. However, effects of caffeine were generally only diminished at doses of (−)-PIA that decreased overall response rates when administered alone. Furthermore, under the schedule of electric-shock presentation increases in response rates produced by some combinations of caffeine and (−)-PIA were as large as those observed after administration of caffeine alone, even with doses of (−)-PIA that decreased response rates when administered alone. Similar results were obtained previously under fixed-interval schedules of food presentation or termination of a stimulus and accompanying electric-shocks in squirrel monkeys (Glowa and Spealman, 1984) and under fixed-interval schedules of food presentation in rats (Goldberg *et al.*, 1985). However, under one procedure increases in rates of schedule-controlled responding produced by caffeine were attenuated by coadministration of doses of (−)-PIA that were inactive when administered alone (Glowa and Spealman, 1984). Under that procedure, responding was maintained by termination of a stimulus accompanied by intermittent electric shocks and punished by occasional response-produced electric shocks. With the above punishment procedure as an exception, increases in rates of responding produced by caffeine generally are only attenuated, if at all, by doses of (−)-PIA that by themselves decrease response rates.

Studies of methylxanthine effects other than increases in rates of behavior generally have not found the effects to be antagonized by adenosine analogs. For example, the decreases in rates of schedule-controlled responding produced by caffeine in the present study, and in others (*e.g.* Glowa and Spealman, 1984; Glowa *et al.*, 1985, Goldberg *et al.*, 1985), were not attenuated by coadministration of (−)-PIA. Additionally, the convulsant effects of the methylxanthine, theophylline, were not antagonized by (−)-PIA (Dunwiddie and Worth, 1982). In subjects trained with caffeine as a discriminative stimulus, (−)-PIA did not antagonize the discriminative effects of caffeine (Holloway *et al.*, 1985). In contrast, in rats trained with (−)-PIA as a discriminative stimulus, caffeine did antagonize the effects of (−)-PIA (Spencer and Lal, 1983). These results as well as the present findings confirm that caffeine can function as an antagonist of the behavioral effects of adenosine-receptor agonists, but provide little evidence for adenosine antagonism as a mechanism for a variety of behavioral effects of caffeine.

Data on the time course of the psychomotor stimulant effects of caffeine and the adenosine antagonist effects also suggest that the two actions are independent. The psychomotor stimulant effects were most pronounced on the session that followed their administration immediately. In contrast, the antagonist effects of caffeine were relatively long acting. Adenosine antagonist actions were observed up to 48 hr after caffeine administration. Slow elimination of caffeine in squirrel monkeys has been observed previously (Burg, 1975). Although the present data are suggestive of the independence of the two effects of caffeine, it is possible that tachyphylaxis developed to the psychomotor stimulant effects of caffeine that were due to an adenosine receptor antagonist action.

With the daily administration of caffeine, tolerance developed to the increases in rates of operant responding produced by caffeine. Previous studies have shown tolerance to the increases in locomotor activity produced by caffeine (Holtzman, 1983: Ahlijanian and Takemori, 1986). Chronic administration of caffeine has also been shown to produce changes in number, but not affinity, of adenosine receptors (*e.g.*, Fredholm, 1982; Ahlijanian and Takemori, 1986). Accordingly, there have also been reports of supersensitivity to actions of adenosine analogs after chronic caffeine administration. For example, Ahlijanian and Takemori (1986) found an increase in potency of (−)-PIA as a depressant of locomotor activity. Others have found an increase in potency of adenosine for producing hypotension (von Borstel *et al.*, 1983). In contrast, the present study showed (−)-PIA dose-effect curves shifted to the right by chronic

caffeine administration. Inasmuch as the antagonist effects of caffeine were apparent for relatively long duration, washout periods of 24 and 48 hr were given before tests of (−)-PIA effects. Even with washout periods of 48 hr there was no indication of sensitivity to the effects of (−)-PIA on response rates in the present study.

One result of the study of daily caffeine administration was an indication that tolerance did not develop to the adenosine-antagonist actions of caffeine. The shifts to the right in the (−)-PIA dose-effect curves during the daily caffeine administration approximated those obtained with acute caffeine and (−)-PIA combination. Independence of the psychomotor-stimulant effects and adenosine antagonist actions of caffeine are supported further by the findings of tolerance to the increases in operant responding without appreciable changes in the antagonism of (−)-PIA during daily caffeine administration.

When two drugs given alone have opposing behavioral effects, the effects of the two drugs combined may be the result of physiological antagonism. Because in the present study antagonism of the effects of caffeine generally did not occur at any doses of (−)-PIA that were inactive alone, the attenuation of effects of caffeine only at active doses of (−)-PIA may be due to the opposing effects of the two drugs. Therefore, the present study examined the similarity of the interactions of caffeine with (−)-PIA and caffeine with chlorpromazine, another behaviorally active drug producing decreases in response rates. As was found with (−)-PIA, the increases in response rates produced by caffeine were attenuated by coadministration of chlorpromazine at doses of chlorpromazine that alone decreased response rates. Furthermore, if the rate decreasing effects of (−)-PIA account for its effectiveness in attenuating the effects of caffeine, then (−)-PIA should attenuate the increases in response rate produced by other psychomotor stimulants similarly to the manner in which it attenuated the effects of caffeine. Increases in response rates produced by (+)-amphetamine were attenuated by doses of (−)-PIA that had response-rate decreasing effects of their own. In general, the shapes of the dose-effect curves for combinations of caffeine and (−)-PIA were not appreciably different from the dose-effect curves for combinations of caffeine with chlorpromazine or for combinations of (+)-amphetamine with (−)-PIA. These data suggest that instances of antagonism of the behavioral effects of caffeine by (−)-PIA are due to a physiological antagonism and that although caffeine may be an effective antagonist of the effects of adenosine-receptor agonists, the psychomotor stimulant effects of caffeine may not be due to antagonist actions at adenosine receptors.

Acknowledgments

The authors would like to thank José A. Prada for his expert assistance in all phases of this study. The advice of Dr. D. Bruce Vaupel is greatly appreciated. Additionally, Ms. Donna Shelton and Ms. Margaret Hawkes provided help in preparation of the manuscript.

References

AHLIJANIAN, M. K. AND TAKEMORI, A. E. Cross-tolerance studies between caffeine and (−)-N^6-(phenylisorpropyl)-adenosine (PIA) in mice. Life Sci. **38:** 577–588, 1986.

BARRETT, J. E.: Behavioral pharmacology of the squirrel monkey. *In* Handbook of Squirrel Monkey Research, ed. by L. A. Rosenblum and C. L. Coe, pp. 315–348, Plenum Publishing Corporation, New York, 1985.

BURG, A. W.: Physiological disposition of caffeine. Drug Metab. Rev. **4:** 1073–1092, 1975.

COFFIN, V. L. AND CARNEY, J. M.: Behavioral pharmacology of adenosine analogs. *In* Physiology and Pharmacology of Adenosine Derivatives, ed. by J. W. Daly, Y. Kuroda, J. W. Phillis, H. Shimizu and M. Ui, pp. 267–274, Raven Press, New York, 1983.

COFFIN, V. L., TAYLOR, J. A., PHILLIS, J. W., ALTMAN, H. J. AND BARRACO, R. A.: Behavioral interaction of adenosine and methylxanthines on central purinergic systems. Neurosci. Lett. **47:** 91–98, 1984.

DAVIS, T. R. A., KENSLER, C. J. AND DEWS, P. B.: Comparison of behavioral effects of nicotine, *d*-amphetamine, caffeine and dimethylheptyl tetrahydrocannabinol in squirrel monkeys. Psychopharmacologia **32:** 51–65, 1973.

DEWS, P. B.: The measurement of the influence of drugs on voluntary activity in mice. Br. J. Pharmacol. **8:** 46–48, 1953.

DUNWIDDIE, T. V. AND WORTH T.: Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J. Pharmacol. Exp. Ther. **220:** 70–76, 1982.

FERSTER, C. B. AND SKINNER, B. F.: Schedules of Reinforcement, Appleton Century Crofts, Inc., New York, 1957.

FREDHOLM, B. B.: Adenosine actions and adenosine receptors after 1 week treatment with caffeine. Acta Physiol. Scand. **115:** 283–286, 1982.

GLOWA, J. R., SOBEL, E., MALASPINA, S. AND DEWS, P. B.: Behavioral effects of caffeine, (−)N-((R)-1-methyl-2-phenylethyl)-adenosine (PIA), and their combination in the mouse. Psychopharmacology **87:** 421–424, 1985.

GLOWA, J. R. AND SPEALMAN, R. D.: Behavioral effects of caffeine, N^6-(L-phenylisopropyl) adenosine and their combination in the squirrel monkey. J. Pharmacol. Exp. Ther. **231:** 665–670, 1984.

GOLDBERG, S. R., KATZ, J. L. AND RISNER, M. E.: Behavioral pharmacology of licit drugs in experimental animals. *In* Problems of Drug Dependence, 1985, ed. by L. S. Harris, NIDA Research Monograph Series, U.S. Government Printing Office, Washington, DC, pp. 36–44, 1986.

GOLDBERG, S. R., PRADA, J. A. AND KATZ, J. L.: Stereoselective behavioral effects of N^6-phenylisopropyl-adenosine and antagonism by caffeine. Psychopharmacology **87:** 272–277, 1985.

HAKE, D. F., AND AZRIN, N. H.: An apparatus for delivering pain shock to monkeys. J. Exp. Anal. Behav. **6:** 297–298, 1963.

HOLLOWAY, F. A., MODROW, H. E. AND MICHAELIS, R. C.: Methylxanthine discrimination in the rat: Possible benzodiazepine and adenosine mechanisms. Pharmacol. Biochem. Behav. **22:** 815–824, 1985.

HOLTZMAN, S. G.: Complete, reversible, drug-specific tolerance to stimulation of locomotor activity by caffeine. Life Sci. **33:** 779–787, 1983.

KATIMS, J. J., ANNAU, Z. AND SNYDER, S. H.: Interactions in the behavioral effects of methylxanthines and adenosine derivatives. J. Pharmacol. Exp. Ther. **227:** 167–173, 1983.

KATZ, J.L. AND GOLDBERG, S. R.: Effects of ethylketazocine and morphine on schedule-controlled behavior in pigeons and squirrel monkeys. J. Pharmacol. Exp. Ther. **239:** 433–441, 1986.

KELLEHER, R. T.: Psychomotor stimulants. *In* Drug Abuse: The Clinical and Basic Aspects, ed. by S. N. Pradhan and S. N. Dutta, pp. 116–147, C.V. Mosby Co., St. Louis, 1977.

LOGAN, L. AND CARNEY, J. M.: Antagonism of the behavioral effects of L-phenylisopropyladenosine (L-PIA) by caffeine and its metabolites. Pharmacol. Biochem. Behav. **21:** 375–379, 1984.

MCKEARNEY, J. W.: Maintenance of responding under a fixed-interval schedule of electric shock-presentation. Science (Wash. DC) **160:** 1249–1251, 1968.

SATTIN, A. AND RALL, T. W.: The effect of adenosine and adenine nucleotides on the cyclic adenosine 3′,5′-phosphate content of guinea pig cerebral cortex slices. Mol. Pharmacol. **6:** 13–23, 1970.

SIROCHMAN,V. AND CARNEY, J. M.: Behavioral pharmacology of adenosine analogs. Fed. Proc. **40:** 294, 1981.

SKINNER, B. F. AND HERON, W. T.: Effects of caffeine and benzedrine upon conditioning and extinction. Psychol. Rec. **1:** 340–346, 1937.

SNEDECOR, G. W. AND COCHRAN, W. G.: Statistical Methods, 6th ed., pp. 135–171, Iowa State University Press, Ames, Iowa, 1967.

SNYDER, S. H., KATIMS, J. J., ANNAU, Z., BRUNS, R. F. AND DALY, J. W.: Adenosine receptors and behavioral actions of methylxanthines. Proc. Natl. Acad. Sci. U.S.A. **78:** 3260–3264, 1981.

SPENCER, D. G. AND LAL, H.: Discriminative stimulus properties of *L*-phenylisopropyl adenosine: Blockade by caffeine and generalization to 2-chloroadenosine. Life Sci. **32:** 2329–2333, 1983.

VON BORSTEL, R. W., WURTMAN, R. J. AND CONLAY, L. A.: Chronic caffeine consumption potentiates the hypotensive action of circulating adenosine. Life Sci. **32:** 1151–1158, 1983.

Send reprint requests to: J. L. Katz, Ph.D., National Institute on Drug Abuse Addiction Research Center, P.O. Box 5180, Baltimore, MD. 21224.