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Effects of Adenosine Analogs Alone and in Combination With Caffeine in the Squirrel Monkey

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KATZ, J. L., J. A. PRADA AND S. R. GOLDBERG. *Effects of adenosine analogs alone and in combination with caffeine in the squirrel monkey.* PHARMACOL BIOCHEM BEHAV **29**(2) 429–432, 1988.—Effects of the adenosine analogs R-N^6-phenylisopropyl-adenosine (R-PIA) and 5'-N-ethylcarboxamidoadenosine (NECA), alone and in combination with caffeine, were studied in squirrel monkeys trained to respond under multiple fixed-interval fixed-ratio schedules of reinforcement. Both drugs produced dose-related decreases in rates of responding, with little difference between effects in the two components. NECA was about ten times more potent than R-PIA in producing these effects, the order of potency suggests that these effects may be mediated by actions at A_2-adenosine receptors. Effects of either drug were antagonized by caffeine. Caffeine when administered alone increased rates of responding. The increases in response rates produced by caffeine were altered by R-PIA only at doses of R-PIA that alone decreased response rates. Effects of caffeine were either enhanced or attenuated by doses of NECA that were inactive when administered alone. These results do not support the notion that increases in rates of behavior, e.g., psychomotor-stimulant effects, produced by caffeine are due to its antagonist actions at adenosine receptors.

Caffeine Adenosine analogs Psychomotor stimulant effects 5'-N-ethylcarboxamidoadenosine (NECA) R-N^6-phenylisopropyl-adenosine (R-PIA) Physiological antagonism

RECENTLY, the focus of studies on the mechanism of action of caffeine have shifted from phosphodiesterase inhibition to antagonist actions at adenosine receptors. For some time it has been known that effects of adenosine can be antagonized by various methylxanthines, including caffeine [16]. More recently, Snyder *et al.* [18] showed that the concentrations of various methylxanthines in brain after doses that increased locomotor activity were below the concentrations needed to produce phosphodiesterase inhibition, and further, the order of potency of these methylxanthines in displacing a labeled adenosine analog from mouse-brain membranes corresponded to the order of potency for increasing locomotor activity. This study also showed that decreases in locomotor activity produced by the adenosine analog R-PIA could be reversed by various methylxanthines including caffeine. A number of following studies substantiated that methylxanthines, particularly caffeine, could antagonize behavioral effects of adenosine analogs, most notably R-phenylisopropyl-adenosine. Studies by Sirochman and Carney [17] showed that effects of R-PIA on operant responding maintained under fixed-ratio schedules were antagonized by caffeine.

Caffeine, like other psychomotor-stimulants [13], increases rates of operant responding as well as locomotor activity [6,15]. Several studies have examined the alteration by R-PIA of the effects of caffeine on schedule-controlled responding maintained under fixed-interval schedules of reinforcement [8–10, 12]. Under fixed-interval schedules, the first response after the lapse of a fixed period of time is reinforced. Generally, studies have shown that caffeine increased rates of responding under fixed-interval schedules. In addition, the increases in response rates produced by caffeine were attenuated by concurrent administration of the adenosine analog R-PIA. However, the attenuation of effects of caffeine were typically obtained only at doses of R-PIA that decreased response rates when administered alone.

The present study was an extension of these earlier studies to the adenosine analog, 5'-N-ethylcarboxamidoadenosine (NECA). This compound has actions at both A_1- and A_2-adenosine receptors but is more potent than R-PIA only at A_2-adenosine receptors [3,5].

METHOD

Subjects

Four adult male squirrel monkeys *(Saimiri sciureus)* served as subjects, and were food deprived to 85–90% of their ad lib body weights. The daily food ration (Purina Monkey Chow supplemented with Teklad Monkey Diet) was adjusted to maintain those body weights throughout the course of the study. 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 various drugs but not more frequently than once per week.

Apparatus

During experimental sessions, subjects were seated and

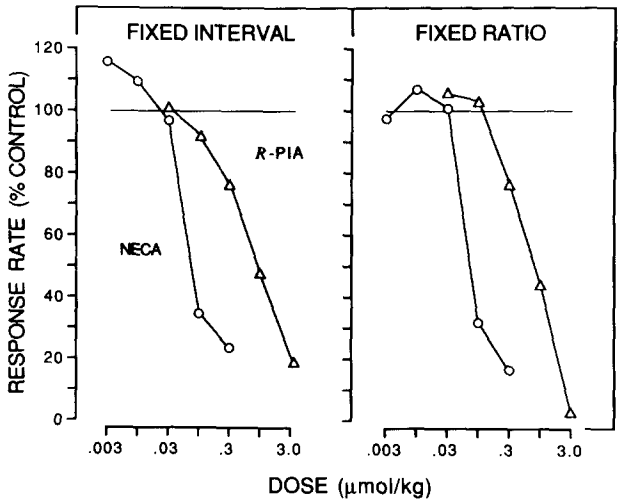

FIG. 1. Effects of R-PIA and NECA on average rates of responding in squirrel monkeys under the fixed-interval fixed-ratio schedule of food presentation. *Ordinates:* averages response rates expressed as a percentage of control response rates; *Abscissae*: dose of drug in μmol/kg, log scale. *Filled circles:* effects of NECA; *Triangles*: effects of R-PIA. Note that NECA was about ten times more potent than R-PIA.

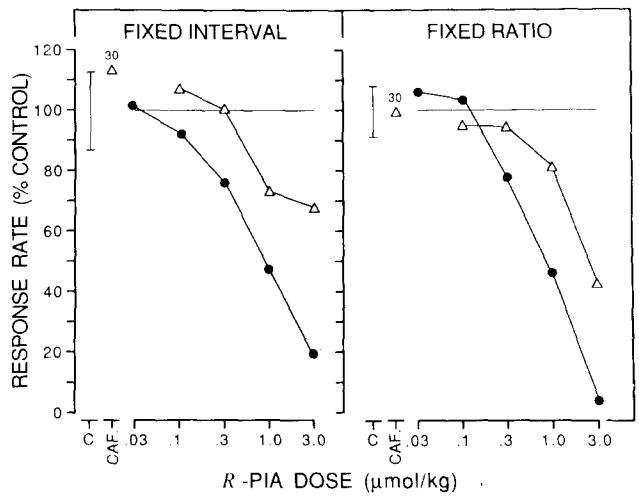

FIG. 2. Effects of R-PIA alone and in combination with 30.0 μmol/kg of caffeine on responding in squirrel monkeys under the multiple fixed-interval fixed-ratio schedule of food presentation. *Filled points:* effects of R-PIA alone; *Open triangles:* effects of R-PIA in combination with caffeine. Other details as in Fig. 1. Note that caffeine shifted the R-PIA dose-effect curve about one log unit to the right.

restrained loosely about the waist in Plexiglas chairs [2,11] which were placed within ventilated, sound-attenuating chambers (Model AC-3, Industrial Acoustics Co., Bronx, NY). The chambers were provided with continuous white noise to mask extraneous sounds. Mounted on the front panel of each chair was a response key (Model 121-09, 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 were three pairs of stimulus lamps (7 W, 120 V AC) which were colored differently and could be individually illuminated. 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.

Behavioral Procedures

Key-press responding was maintained under a multiple fixed-interval fixed-ratio schedule of food presentation during experimental sessions that were conducted daily, five days per week. During sessions in the presence of green stimulus lamps, the first response after the lapse of 5 min produced a food pellet accompanied by the extinguishing of the green lamps and a 200-msec flash of white stimulus lamps (fixed-interval 5-min schedule). A 60-sec timeout period followed each food presentation during which all stimulus lamps were out and responding had no scheduled consequences. Following the timeout, red stimulus lamps were illuminated and the thirtieth response produced food presentation, turned off the red lights, produced the 200-msec flash of white lights (fixed-ratio 30 schedule), and was followed by the 60-sec timeout. If a response did not occur within one minute after the lapse of the 5-minute fixed-interval or the onset of the fixed-ratio component, the timeout followed without food delivery. Sessions ended after the twentieth timeout period.

Drugs and Injection Procedures

The base forms of R-PIA (Boehringer Mannheim, Indianapolis, IN) and NECA (RBI, Wayland, MA) were dissolved in 0.1 N HCl and diluted with saline (0.9% NaCl) to achieve the appropriate concentration. Caffeine sodium benzoate (Sigma, St. Louis, MO) was dissolved in saline. Doses were injected intramuscularly (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 IM (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 μmol per kg 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 once or twice in each subject in a mixed sequence with a complete dose-effect curve determined before another drug or drug combination was studied.

Measurement of Effects

Overall rates of responding for individual subjects were computed each session by dividing total responses by elapsed time. Effects of each drug or drug combination are expressed as the overall rate of responding as a percentage of the mean response rate from all vehicle-control sessions. Effects of the drugs shown in the figures are the means for all subjects.

RESULTS

Performances under the multiple schedule were similar to

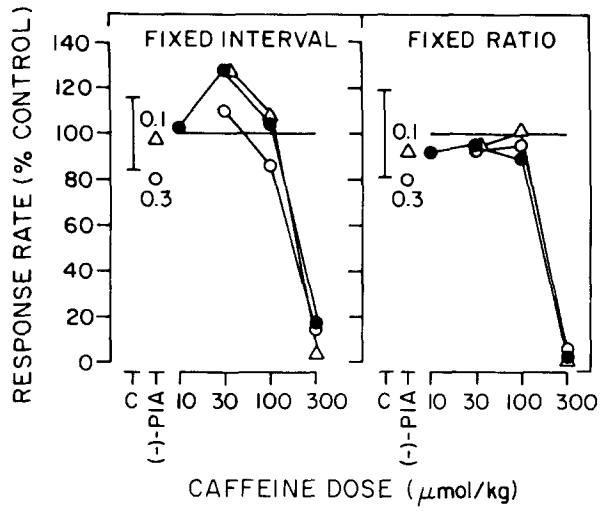

FIG. 3. Effects of caffeine alone and in combination with R-PIA on responding in squirrel monkeys under the multiple fixed-interval fixed-ratio schedule of food presentation. *Filled points:* caffeine alone; *Open triangles:* caffeine with 0.1 μmol/kg R-PIA; *Open circles:* caffeine with 0.3 μmol/kg R-PIA. Other details as in Fig. 1. Note that increases in response rates produced by caffeine were only altered by doses of R-PIA that had effects when administered alone.

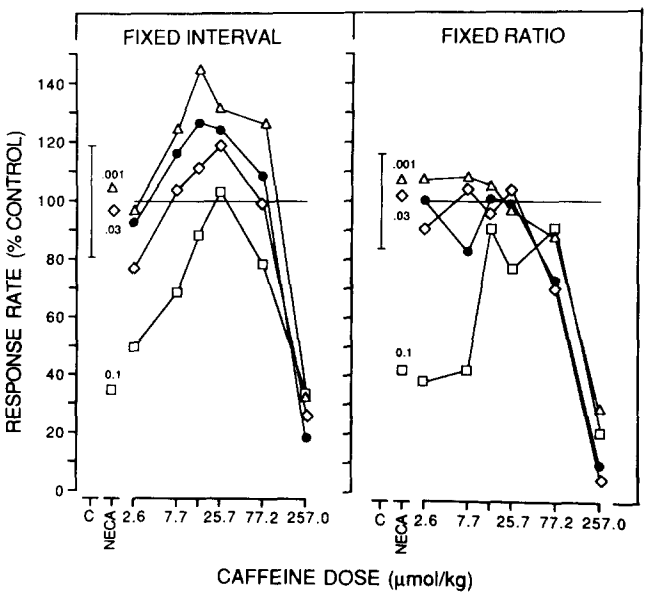

FIG. 4. Effects of caffeine alone and in combinations with NECA on responding under the multiple fixed-interval fixed-ratio schedule of food presentation in squirrel monkeys. *Filled points:* caffeine alone; *Open triangles:* caffeine with 0.001 μmol/kg NECA; *Open diamonds:* caffeine with 0.03 μmol/kg NECA; *Open squares:* caffeine with 0.1 μmol/kg NECA. Other details as in Fig. 1. Note that increases in response rates produced by caffeine were enhanced or attenuated by doses of NECA that were inactive when administered alone.

those obtained previously under similar schedules [7]. Under the fixed-interval schedule, there was an initial low response rate that increased with time up to food presentation. Under the fixed-ratio schedule, responding occurred at a high rate up to food presentation. Little or no responding occurred during the timeout periods.

Both R-PIA and NECA produced dose-related decreases in rates of responding in both the fixed-interval and fixed-ratio components of the schedule. With both drugs there was little or no difference between the effects on rates of responding in the two components; rates of responding were decreased by a dose of 0.1 μmol/kg of NECA. Comparable decreases in response rates produced by R-PIA required doses greater than ten times higher; the dose-effect curves for NECA were about one log unit to the left of those for R-PIA (Fig. 1).

Effects of R-PIA on response rates were antagonized by caffeine. Concurent administration of 30.0 μmol/kg of caffeine shifted the R-PIA dose-effect curve to the right by about one half log unit in both components of the schedule (Fig. 2). This dose of caffeine when administered alone increased rates of responding under the fixed-interval schedule but had no effect on rates of responding under the fixed-ratio schedule.

Intermediate doses of caffeine (14.4 to 30.0 μmol/kg) increased response rates during the fixed-interval schedule; at the same dose there was no effect on responding under the fixed-ratio schedule (Figs. 3 and 4; filled symbols). At the highest doses (257, 300 μmol/kg), response rates under both schedules were decreased by caffeine.

The lowest dose of R-PIA (0.1 μmol/kg) studied in combination with caffeine had no effects on responding when administered alone. When administered with caffeine, the effects of caffeine were not appreciably altered (Fig. 3; open triangles). A higher dose of R-PIA (0.3 μmol/kg), that slightly decreased response rates when administered alone, attenuated the increases in response rates produced by caffeine (Fig. 3; open circles). Decreases in response rates produced by the higher doses of caffeine under either schedule were not appreciably altered by either dose of R-PIA.

The lowest doses of NECA (0.001, 0.03 μmol/kg) studied in the combination with caffeine had no effects on responding when administered alone. When administered with caffeine, the lower of the two doses slightly enhanced the increases in response rates produced by caffeine (Fig. 4; open triangles); the higher of the two doses slightly attenuated the increases in response rates produced by caffeine (Fig. 4; open diamonds). A higher dose of NECA (0.1 μmol/kg), that slightly decreased response rates when administered alone, attenuated the increases in response rates produced by caffeine (Fig. 4; open circles). Decreases in response rates produced by the higher doses of caffeine under either schedule were not appreciably altered by either dose of NECA.

Antagonism of the effects of NECA by caffeine could be seen at the combination of the highest dose with caffeine (Fig. 4; open squares). That dose of NECA, when administered alone, decreased response rates in either component to between 30 and 40 percent of control. Caffeine dose-dependently antagonized the decreases in rates produced by NECA.

DISCUSSION

In the present study, both NECA and R-PIA produced dose-related decreases in response rates. NECA was about ten times more potent than R-PIA. Similar potency relations have been reported previously for the two drugs for effects on locomotor activity in mice [1] and for effects on operant

behavior in rats [14]. The order of potency for the two drugs is similar to the order of potency for the two drugs for stimulating adenylate cyclase activity [5] and for displacing [^{3}H]NECA (in the presence of cold N^6-cyclopentyl-adenosine) from rat striatal membranes, suggesting that the behavioral effects of the two drugs may be mediated by actions at A_2-adenosine receptors.

Previous studies of behavioral effects of adenosine analogs [4, 10, 17, 18] have found that R-PIA is more potent than S-PIA. Since R-PIA is much more potent than S-PIA in producing effects mediated by A_1-adenosine receptors (such as evidenced by inhibition of adenylate cyclase, e.g., [5]), the results of the previous behavioral studies suggested that the behavioral effects of the drugs were mediated by actions at A_1-adenosine receptors [10]. However, R-PIA is also more potent than S-PIA in producing effects mediated by A_2-adenosine receptors [5]. Further, in studies with squirrel monkeys, Spealman and Coffin [19] found a better correlation of potencies for producing behavioral effects of a series of adenosine analogs with K_i values (obtained from [3]) for displacing bound [^{3}H]NECA than for displacing [^{3}H]CHA from rat brain membranes, suggesting that the effects were mediated by A_2-adenosine receptors. In contrast, the rank order of potencies of a series of adenosine analogs on schedule-controlled behavior in rats was closely related to the rank order of potencies for inhibiting binding to A_1-adenosine receptors [4]. The present results are consistent with those of Spealman and Coffin [19] in suggesting that the behavioral effects of adenosine analogs are mediated by A_2-adenosine receptors.

As has been found in several previous studies [6, 9, 12, 15], modest increases in response rates under the fixed-interval schedule were produced by caffeine administration. Additionally, the increases in response rates were attenuated by the co-administration of the adenosine analogs. However, increases in response rates were only attenuated at doses of R-PIA that decreased rates of responding when administered alone. Similar results, as well as similar results with interactions of caffeine and chlorpromazine, suggested that the antagonism of psychomotor stimulant effects of caffeine by R-PIA was the result of physiological antagonism [12].

These conclusions do not suggest that caffeine is not an effective antagonist of the actions of adenosine. Indeed, the present results are in accord with others showing effective antagonism of the effects of adenosine analogs by caffeine. However, while caffeine may effectively antagonize effects of adenosine analogs, and possibly endogenous adenosine, the present results suggest that an antagonist action at adenosine receptors is not the mechanism for certain behavioral effects of caffeine, including the increases in rates of operant responding.

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