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Antagonism of the Behavioral Effects of L-Phenylisopropyladenosine (L-PIA) by Caffeine and its Metabolites[1]

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LOGAN, L. AND J. M. CARNEY. *Antagonism of the behavioral effects of L-phenylisopropyladenosine (L-PIA) by caffeine and its metabolites.* PHARMACOL BIOCHEM BEHAV 21(3) 375–379, 1984.—Three male Sprague-Dawley strain rats were trained to respond under a multi-component time out 5 min variable ratio 15 (VR15) schedule of food reinforcement. Cumulative, within session dose-effect curves were determined for L-PIA alone and after methylxanthine pretreatment. L-PIA alone produced dose related decreases on VR15 responding at doses between 0.032 and 0.178 mg/kg. Significant antagonism of L-PIA was demonstrated from pretreatment with caffeine, theophylline, theobromine, paraxanthine, 3-methylxanthine, and 7-methylxanthine. No antagonism of L-PIA was observed following pretreatment with 1-methylxanthine. Consistent with the adenosine receptor blockade hypothesis, caffeine also antagonized the effects of 5'-N-ethylcarboxamide adenosine (NECA) on VR15 responding.

Caffeine L-PIA NECA Methylxanthines Variable ratio

METHYLXANTHINES are widely consumed for their presumed psychotropic effects. In addition, they are prescribed for a variety of therapeutic purposes. For example, both caffeine and theophylline can be used to treat apnea of preterm infants [1,12]. Theophylline is the most common drug used in the daily treatment of asthma [8]. Vasodilatory headaches are often treated with caffeine (T. L. Whitsett, personal communication). It has been demonstrated that many of caffeine's pharmacological effects involve the competitive blockade of tissue adenosine receptors [6,9]. This blockade occurs at relatively low xanthine concentrations. At higher concentrations, methylxanthines can inhibit cyclic nucleotide phosphodiesterase [2,13] and 5'-nucleotidases [11]. In contrast, the central nervous system stimulant effects of caffeine occur at relatively low doses [9]. Wu *et al.* [14] recently reported it may be possible to modify these activities by altering the xanthine structure [1]. For example, a series of 8-substituted theophylline analogs were much more potent as adenosine antagonists than as phosphodiesterase inhibitors. In contrast, substituted xanthines with increased lipid solubility (e.g., 1,3,7 trimethyl-6-thioxo-2-purine) were essentially equipotent as inhibitors of phosphodiesterase and adenosine receptors [14].

The effects of adenosine and its structural analogs on various *in vitro* and *in vivo* systems have been described [5]. Adenosine and its analogs have been demonstrated to produce a variety of CNS effects. These effects include: depression of neuronal firing rates [6], decreases in locomotor activity [9], increase in slow wave sleep [7] and disruption of schedule-controlled responding [3]. All of these effects can be reversed by the administration of caffeine. This study was conducted as the first in a series of structure activity studies on the interaction of adenosine agonists and methylxanthines, using operant behavior. Caffeine can be metabolized to dimethylxanthines (paraxanthine, theophylline and theobromine) and further metabolized to 1-methyl, 3-methyl, and 7-methylxanthine. All of these xanthines have been shown to interact with the different adenosine receptors [9]. While these metabolites have been identified in humans and laboratory subjects, the relative contribution of the metabolites to the observed effects following caffeine administration has not been determined. The present study was designed to determine if caffeine, the dimethylxanthine and the monomethylxanthine metabolites all functioned as antagonists of the behavioral effects of adenosine receptor agonists.

METHOD

Subjects

Three male Sprague-Dawley rats, weighing 300–375 g were used in the study. The subjects were individually housed in suspended stainless steel cages equipped with an automatic watering system. Ambient room temperature was 22°C, and the room was kept on a 12 hr light/12 hr dark cycle. The rats were food deprived to 80% of their free-feeding

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weight prior to initiation of the study. All training and experimental sessions were conducted during the morning hours (0800–1200).

Procedure

The testing apparatus consisted of an experimental operant chamber (Colbourn Instruments) placed in a sound attenuating box. A response lever was located on the left side of the front wall of the chamber. Three lights were present above the lever, illumination of the center yellow light served as the discriminative stimulus for the food reinforced component of the test schedule. Food pellets (45 mg, Bio Serv) were delivered by a pellet dispenser into a trough located in the center of the front wall. Schedule conditions were controlled by solid-state programming equipment. The number of responses and reinforcements were recorded on digital counters and a cumulative chart recorder provided a graphical representation of responding.

The rats were trained to respond for food pellets by reinforcing each lever press with a single food pellet. Once the rats were reliably responding for a single food pellet per response, the schedule was changed from continuous reinforcement to a multi-component variable ratio schedule (VR). Under the VR schedule each subject was required to respond an average of 15 times (VR 15) for a single food pellet. The number of responses necessary to obtain a food pellet varied from 5 to 25. Test sessions consisted of alternating time out components and active VR components, each component was of 5 minutes duration. This schedule was a modification of those reported by Coffin and Carney [3]. During the time out period responses were recorded but not reinforced, responses were recorded and food reinforced during the VR component. The onset of the VR component was signaled by illumination of the center yellow light. Rats were allowed to adapt to the test chamber for 5 minutes prior to the first time out period. Sessions were run 5 or 6 times a week, and approximately 5 weeks elapsed before a stable rate and pattern of responding developed across components and on a day to day basis.

Drug Testing

Initial dose effect curves for L-PIA were determined following acquisition of stable responding. Cumulative doses of L-PIA (0.01, 0.03, 0.10, 0.178 mg/kg) were given by intraperitoneal injection at the beginning of each time out period (0, 10, 20, 30 min). The effects of the methylxanthines on L-PIA induced decreases in responding were determined by administering the methylxanthines intraperitoneally 20 minutes prior to the first time out period, and cumulative doses of PIA were given as described. Drug sessions were conducted no more than twice weekly.

Drugs

The drugs and dosages used in this study were L-N6-phenylisopropyl adenosine (L-PIA, 0.01, 0.03, 0.10, 0.178 mg/kg cumulative dosing); caffeine (1.0, 3.2, 10.0 mg/kg); theophylline (3.2 mg/kg); paraxanthine (3.2 mg/kg); theobromine (10.0 mg/kg); 1-methylxanthine, 3-methylxanthine, and 7-methylxanthine, each at 32 mg/kg. The series of the drugs used were as follows: L-PIA (Boehringer Mannheim Biochemical); 5'-N-ethylcarboxamide adenosine (NECA) (Dr. J. Bristol, Warner Lambert/Parke Davis Pharmaceutical); caffeine, theophylline, theobromine (Eastman Kodak Co.); paraxanthine, 1-methylxanthine, 3-methylxanthine, 7-methylxanthine (Adams Chemical Co., Round Lake, IL). All doses refer to the base.

TABLE 1

CONTROL BASELINE PERFORMANCE OF RATS RESPONDING UNDER THE MULTICOMPONENT TIME OUT 5 MIN (TO) VARIABLE RATIO 15 (VR15) SCHEDULE

Rat No.	TO (resp/5 min)	VR 15 (resp/sec)
408	26 (±11)	1.78 (±0.04)
	36 (±9)	1.88 (±0.08)
	65 (±14)	1.80 (±0.06)
	47 (±14)	1.70 (±0.08)
409	24 (±9)	1.96 (±0.08)
	32 (±13)	2.08 (±0.10)
	50 (±24)	2.02 (±0.08)
	26 (±12)	1.88 (±0.06)
410	8 (±3)	1.50 (±0.06)
	8 (±3)	1.70 (±0.06)
	6 (±2)	1.70 (±0.06)
	5 (±2)	1.62 (±0.06)

Each value is the mean of 8 control sessions (±S.E.).
VR responding is presented as responses/second and time out responding is presented as responses per 5 min.

Statistics

Variable ratio and time out component responding were individually calculated and expressed as responses/second. The effects of PIA alone and following pre-session methylxanthine injection was calculated as a percentage of the animals own control performance. Dose-effect curves were determined and expressed as the mean (± standard error) for the doses tested. Dose-effect curves were compared using a two-way analysis of variance.

RESULTS

VR responding under the multicomponent schedule occurred at a relatively high and constant rate of responding (Table 1). The average rate of responding for the three subjects was 1.78, 1.98, 1.62 resp/sec. Relatively few responses were emitted during the time out component. This level of stimulus control over responding is similar to what has been described for both fixed ratio and differential reinforcement of low rate [3] schedules.

Cumulative, within-session doses of L-PIA produced dose related decreases in VR 15 responding (Fig. 1). Relatively low doses (0.01 and 0.032 mg/kg) produced a slight decrease in VR performance. At 0.1 and 0.178 mg/kg responding was almost completely suppressed. Caffeine produced a dose related antagonism of the L-PIA induced rate decreases (Figs. 1 and 2). Increasing the pretreatment dose from 1.0 to 10.0 mg/kg resulted in greater degrees of L-PIA antagonism. A dose of 10 mg/kg completely blocked the effects of 0.1 mg/kg L-PIA (Fig. 2). Pretreatment with 3.2 mg/kg caffeine resulted in an approximately 3-fold shift to the right of the L-PIA dose effect curve (Fig. 3).

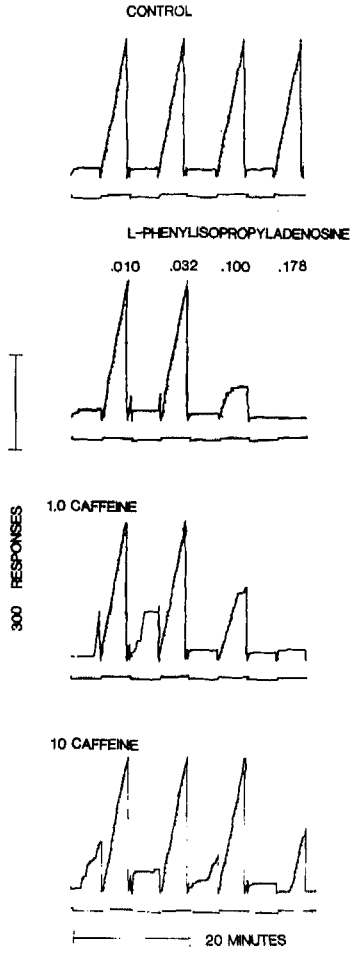

FIG. 1. Representative cumulative records of VR 15 responding for rat No. 408. Top record depicts control performance. Downward deflection of the lower pen denotes time out component (non-reinforced), upward deflection denotes VR 15 component (reinforced). Responding is indicated by upward migration of upper pen. Pen is reset after 500 responses or end of component. Second record depicts dose related decreases in responding produced by cumulative doses of L-PIA. Third and fourth records show antagonism of L-PIA effects on VR 15 responding by 1.0 and 10 mg/kg caffeine, respectively. Caffeine doses were given 20 min prior to first time out component; saline and L-PIA were given at the onset of time out components.

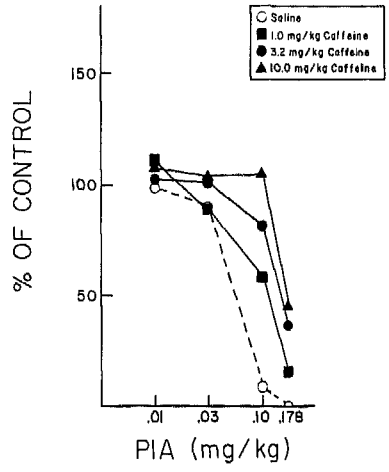

FIG. 2. Antagonism of L-PIA effects on VR 15 responding by caffeine in rat No. 408. Caffeine was given IP 20 minutes prior to first time out component. Cumulative doses of L-PIA were given IP at onset of time out components.

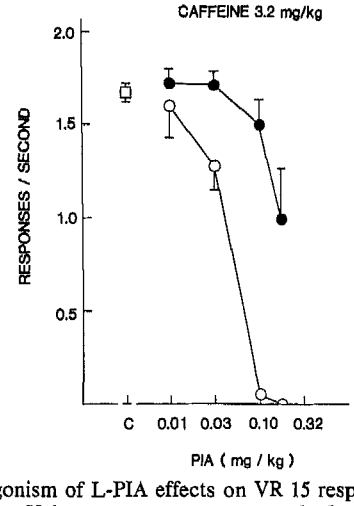

FIG. 3. Antagonism of L-PIA effects on VR 15 responding by 3.2 mg/kg caffeine. Values represent mean±standard error for three subjects. Control valve represents average responding for all components from previous day (saline). Open circles represent saline pretreatment, solid circles represent caffeine pretreatment.

All of the dimethylxanthines tested were effective as L-PIA antagonists (Fig. 4). Of the doses tested, theophylline appeared to be more effective than paraxanthine and theobromine was the least active. Since these dimethylxanthines have similar plasma and brain pharmacokinetics in the rat (unpublished observations), it would appear likely that the rank order for brain adenosine receptor affinity is similar to their behavioral potencies.

The monomethylxanthine metabolites of caffeine also demonstrated significant L-PIA antagonist activity (Fig. 5). Both 3-methylxanthine and 7-methylxanthine produced significant shifts to the right of the L-PIA dose effect curves. Pretreatment with 1-methylxanthine produced variable effects. In some rats there was a suggestion of antagonism, while in other rats there was none. No significant antagonism of L-PIA was demonstrable under the present test condition.

Since caffeine and other methylxanthine have been shown to block the A_1 and the A_2 receptor site for adenosine, antagonism of the A_2 agonist NECA by caffeine was determined. As was the case for PIA, 3.2 mg/kg caffeine shifted the NECA dose-effect curve to the right (Fig. 6). The magnitude of the NECA shift appeared to be equivalent to that seen with L-PIA.

DISCUSSION

Utilization of a variable ratio schedule provides a stable and reliable system for quantifying the behaviorally disruptive effects of a variety of psychoactive compounds. The

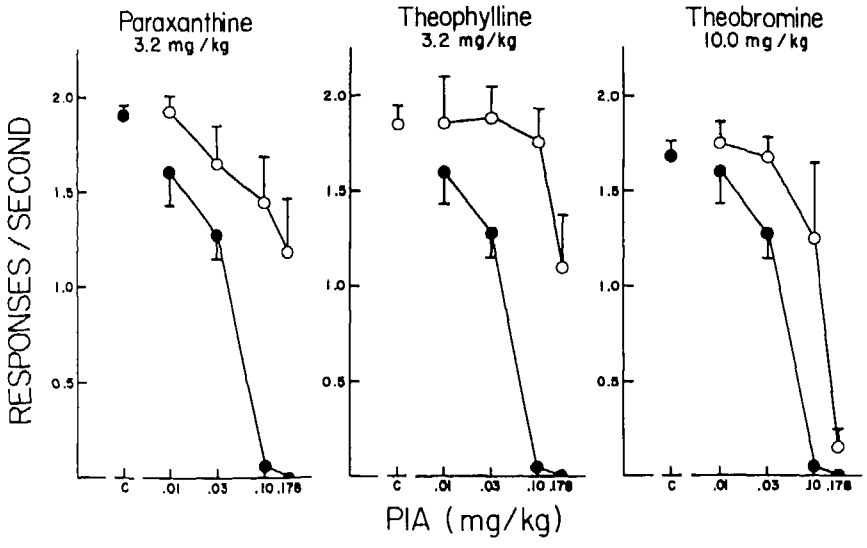

FIG. 4. Dimethylxanthine antagonism of L-PIA effects on VR 15 responding. Values represent mean±standard error for three subjects. Solid circles represent saline pretreatment, open circles represent dimethylxanthine pretreatment.

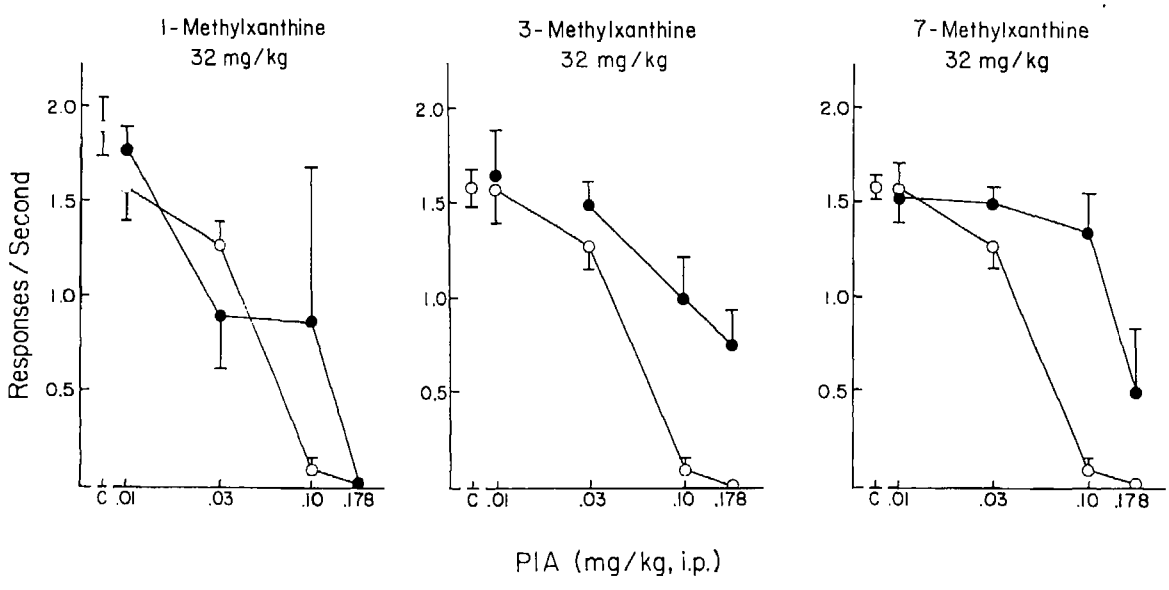

FIG. 5. Monomethylxanthine antagonism of L-PIA effects on VR 15 responding. Values represent mean±standard error for three subjects. Open circles represent saline pretreatment, solid circles represent monomethylxanthine pretreatment.

approximate ED_{50} (0.05 mg/kg) for the disruptive effects of L-PIA is in accord with previous observations [3]. The results of this experiment demonstrate that methylxanthines are capable of antagonizing of the behavioral effects of both L-PIA and NECA.

The ability of caffeine to antagonize the disruptive effects of PIA on operant behavior have been described for both rats [3] and non-human primates [14]. In addition caffeine has been shown to block the discriminative stimulus effects of PIA at doses between 0.32 and 3.2 mg/kg [3,10]. Due to the experimental design, no attempt was made to classify the rank order of potency of the methylxanthines used. In mice, theophylline and paraxanthine are nearly equipotent in inhibiting ^{3}H-cyclohexyl adenosine binding at A_1 receptors. Caffeine and theobromine were less potent. At A_2 receptors labeled with ^{3}H diethylphenylxanthine a similar order of potency is observed. The order of potency for locomotor stimulation in mice is theophylline caffeine=paraxanthine theobromine [5]. In the guinea pig tracheal muscle preparation theophylline and 3-methylxanthine are equipotent; 1-methylxanthine, while less potent in terms of the EC_{50} value is capable of producing maximal relaxation [11]. Theophylline and 3-methylxanthine are equipotent in increasing contractile force in the guinea pig heart, while caffeine and 1-methylxanthine are less effective. While cardiac effects may be due in part to phosphodiesterase inhibition

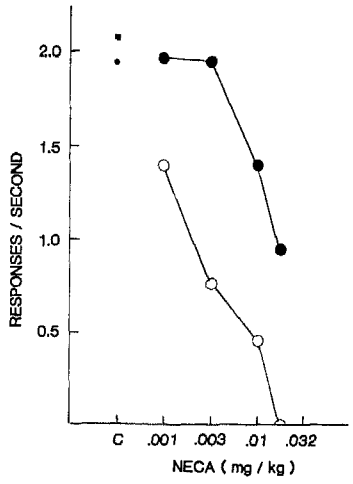

FIG. 6. Antagonism of NECA effects on VR15 responding by 3.2 mg/kg caffeine. Values at C represent the control performance at for the individual rat. Open symbols represent the affect of NECA alone and the solid symbols represent the effects of NECA after 3.2 mg/kg caffeine pretreatment. Each point is the mean of two rats.

the same general potency is observed. Thus, the qualitative effect of caffeine and its metabolites *in vitro* as they relate to adenosine are also seen in their behavioral effects.

The methylxanthines we have investigated are known to be either caffeine or theophylline metabolites and in the intact animal may potentially contribute to the effects of these parent compounds. Factors that would influence the additive effects of these methylxanthines includes their relative potencies and species differences in caffeine or theophylline metabolism, primarily the rate of microsomal demethylation relative to conversion to uric acids by xanthine oxidase, and renal clearance of the metabolic products. The ratio of plasma versus brain concentrations of metabolic products would be an additional factor in studies of the central effects of caffeine or theophylline. Similarly, alterations in methylxanthine metabolism due to neonatal metabolism, pregnancy, alcohol consumption, concurrent drug therapy, etc., may modify the therapeutic response to caffeine or theophylline in humans.

The apparent lack of antagonism by 1-methylxanthine was probably due to a high rate of metabolism. At a cumulative dose of 150 mg/kg, 1-methylxanthine was not detected in plasma, and low levels were detected in brain cortical cup fluid samples (Carney, Christensen and Nakamura, unpublished data). The lack of detectable plasma levels of 1-methylxanthine is due in part to the relatively rapid conversion of 1-methylxanthine to 1-methyluric acid. However, renal clearance factors may also be involved. The high degree of variability present in this study of 1-methylxanthine may be due to individual differences in 1-methylxanthine metabolism.

In addition to the naturally occurring methylxanthines and their metabolic products there exist a variety of substituted xanthine analogs. Some of the compounds possess little or no antagonistic activity at adenosine binding sites and may act as partial adenosinic agonists. In conclusion, these data support the hypothesis that at least part of the behavioral action of methylxanthines is due to blockade of adenosine receptors. In addition, this provides a systematic demonstration of the behavioral effects of caffeine metabolites.

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