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Methylxanthine Discrimination in the Rat: Possible Benzodiazepine and Adenosine Mechanisms[1]

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HOLLOWAY, F. A., H. E. MODROW AND R. C. MICHAELIS. *Methylxanthine discrimination in the rat: Possible benzodiazepine and adenosine mechanisms.* PHARMACOL BIOCHEM BEHAV 22(5) 815–824, 1985.—Rats were trained to discriminate either caffeine or theophylline from saline using a two-lever discrimination paradigm. Since methylxanthines have been found to interfere with agonist binding at both adenosine and benzodiazepine (BDZ) receptors, chlordiazepoxide (CDP) and L-PIA (an adenosine analog) were tested for generalization to and blockade of both xanthine cues. Neither L-PIA nor CDP generalized to either xanthine cue, although both produced dose-related decreases in response rate. CDP, but not L-PIA, produced dose-related decreases in drug-lever responses when combined with training doses of caffeine or theophylline. Response rates indicated a complex interaction between the xanthines and both L-PIA and CDP. When combined with the caffeine training dose, pentobarbital also produced a dose-dependent decrease in response rate but not in drug lever choices. Finally, papaverine generalized to the caffeine cue in a dose-dependent fashion. In a second experiment, rats trained to discriminate CDP from saline showed no generalization in L-PIA tests. CDP-appropriate responding was not significantly affected when the CDP training dose was combined with caffeine. These data indicate that: (a) methylxanthine interactions with L-PIA and CDP on response rate likely involve blockade of adenosine mechanisms; (b) the xanthine cue does not appear to depend on interactions with adenosine receptors; and (c) the xanthine cue may involve effects on cyclic AMP activity and/or interaction with the BDZ/GABA receptor complex.

Methylxanthine Drug discrimination Operant behavior Caffeine Theophylline Chlordiazepoxide L-PIA Phosphodiesterase inhibitor

WHILE it is now established that caffeine can produce a xanthine-specific cue in the rat operant drug-discrimination task [12, 42, 43, 65], the mechanisms mediating this cue are not known. Female Wistar rats trained to detect a 60 mg/kg caffeine cue (water reinforcement on a Fixed Ratio (FR) 10 schedule) showed partial generalization to amphetamine (1.5 mg/kg) and complete generalization to aminophylline [65]. Male Sprague-Dawley rats trained to detect a 32 mg/kg caffeine cue (food reinforcement on a FR10 schedule) showed no generalization to other psychomotor stimulants (e.g., 0.3–2.0 mg/kg amphetamine, 1.0–7.0 mg/kg methylphenidate, 0.1–0.4 mg/kg nicotine, or 10 mg/kg thyrotropin-releasing hormone), but did display dose-related (10–56 mg/kg) generalization to theophylline [43]. Further, comparable groups of rats trained to detect a 32 mg/kg caffeine or a 56 mg/kg theophylline cue (Variable Ratio (VR) 5–15 schedule) showed generalization to the other xanthine training drug [42]. The theophylline-trained rats did not display generalization either to amphetamine or to pentylenetetrazol [42]. The caffeine-trained rats also generalized to several of its xanthine metabolites, including theophylline, paraxanthine, and 3-methylxanthine but not theobromine [14]. Caffeine does generalize to a buproprion discriminative cue (a phenylaminoketone and atypical antidepressant drug), but so do several other stimulant compounds [35]. Thus, although caffeine may share some stimulus feature with other stimulants, the xanthine cue itself appears fairly unique *vis a vis* other psychomotor stimulants.

In considering possible mechanisms which may mediate the discriminative properties of xanthines, a variety of candidates present themselves. For example, caffeine has a number of effects on the synthesis, release, and metabolism of catecholamines and serotonin [3, 4, 5, 45, 56, 64]. However, Winter [65] found that a 60 mg/kg caffeine cue was not blocked by 3 or 10 mg/kg pizotyline (a serotonin antagonist) or by 0.5–2.0 mg/kg spiperone (a dopamine antagonist). Further, rats trained to detect a 32 mg/kg caffeine cue showed no generalization to the dopamine agonist, apomorphine (0.05–0.35 mg/kg), and this caffeine cue was not blocked by haloperidol (0.03 mg/kg) [34]. While not defini-

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tive, the latter data suggest that the xanthine cue is not mediated by dopaminergic or serotonergic mechanisms *per se*.

Several other mechanisms of action have been proposed for the central effects of methylxanthines. For example, methylxanthines inhibit cyclic nucleotide phosphodiesterase, thereby preventing cyclic AMP (c-AMP) destruction and causing c-AMP accumulation [10,15]. However, millimolar concentrations of xanthines are required to inhibit such forms of phosphodiesterase, while low micromolar concentrations of caffeine are required to produce changes in spontaneous activity [59]. Another suggestion is that methylxanthines may alter calcium mobilization and/or distribution [54]. For example, high (156 mg/kg) but not low (20 mg/kg) doses of theophylline have been shown to significantly decrease calcium content in the vesicle-rich subfractions of rat brain cortex [47].

Another proposed mechanism is that methylxanthines interact with the benzodiazepine (BDZ) receptor [41, 46, 58]. Although xanthines have relatively low affinity for BDZ receptors [7], both caffeine and theophylline have been found to competitively inhibit diazepam binding in brain, with caffeine's potency being almost twice that of theophylline [6,41]. While the latter relative xanthine potencies do not match those found for xanthine stimulation effects on locomotor activity [60], they do correlate with those found to produce the caffeine cue [43]. Finally, a variety of xanthine-BDZ interactive effects have been observed, e.g.: (a) certain forms of diazepam have been found to counteract the stimulant effects of caffeine on spontaneous activity in mice [22]; (b) BDZs antagonize caffeine-induced seizures [40]; (c) theophylline antagonizes BDZ-induced depression of cortical neuronal firing [49] and the diazepam-induced release of acetylcholine from rat cerebral cortex [52]; and (d) caffeine has been found to antagonize several other central nervous system effects of diazepam [53]. The principal difficulty with a xanthine-BDZ receptor explanation for xanthine cue effects (see [59,60]) is the apparent differences in caffeine dose ranges necessary to interact with BDZ binding on the one hand (high micromolar; [41]) and to produce the caffeine cue on the other (low micromolar range; [43]).

Finally, one hypothesis recently receiving considerable attention (cf. [59,60]) suggests that many actions of methylxanthines involve antagonism of purine effects at the postulated neuronal receptors for adenosine [9]. Adenosine is an endogenous substrate and potent suppressant of central and behavioral processes [51,59]. A theophylline derivative (1,3-diethyl-8-phenylxanthine) competitively inhibits binding of adenosine analogs to the adenosine receptor [8]. While several species of adenosine receptors have been proposed [39,63], the A1 (but not the A2 type) receptor exhibits stereospecificity [8,61], particularly for the adenosine analog, N6-phenylisopropyladenosine (PIA). Adenosine agonist activity can decrease or increase c-AMP levels. The high affinity A1 receptor inhibits and the low affinity A2 receptor stimulates adenylate cyclase [63]. Further, methylxanthines have now been reported: (a) to block the L-PIA-induced reduction of locomotor activity [61] and of general operant responding [18] as well as the discriminative cue produced by L-PIA [13, 18, 62]; and (b) to prevent adenosine's influences on c-AMP [20,27] and on release of neurotransmitters [17,30] and its depressant effects on neuronal firing rates [51]. Although both xanthines and adenosine agonists can influence c-AMP mechanisms, certain of their effects on behavior appear to be more attributable to adenosine receptor interactions than secondary to effects on c-AMP levels (see [26,28]).

EXPERIMENT 1

RATIONALE

The latter review of candidate mechanisms for the xanthine cue suggests at least two obvious possibilities, i.e., the xanthine cue may depend on xanthine action to antagonize agonist activity through: (a) adenosine and/or (b) BDZ mechanisms. The first study was designed primarily to directly assess these possibilities. Using the two-lever, food-reward operant drug discrimination procedures, separate groups of rats were trained to detect a caffeine or theophylline cue. Blockade of both xanthine cues was assessed by giving the xanthine training dose plus pretreatment with either the adenosine analog L-PIA or chlordiazepoxide (CDP), a moderately potent BDZ. Pretreatment of caffeine-trained rats with the caffeine training dose plus pentobarbital (PBL) was examined as a control for any general sedative effects on caffeine discrimination. Pretreatment of xanthine-trained rats with L-PIA or CDP alone permitted assessment of whether either compound has xanthine cue properties.

Finally, since caffeine is a phosphodiesterase inhibitor, the opportunity presented itself to test a limited sample of caffeine-treated rats for possible generalization to papaverine (PPV), a potent inhibitor of phosphodiesterase [25]. Although PPV is known to inhibit uptake of adenosine by cerebral vasculature [66] and perhaps brain neurons [51], it is not known to have specific interactions with adenosine A1 or A2 receptors *per se*. The net effect of adenosine uptake inhibition, however, is for PPV to potentiate the depressant effects of adenosine on cortical neuronal firing [51] and the adenosine-elicited accumulation of c-AMP in brain slices [33]. Of further interest is the finding that PPV in addition to inhibiting adenosine uptake also competitively inhibits the binding of diazepam to brain membrane BDZ receptors [67].

METHOD

Subjects

Adult male Sprague-Dawley rats (Sasco, Inc.) weighing between 350 and 500 g were used in this study (N=31). Prior to training, the rats were gradually reduced to 80% of their free-feeding weights. Target weights were adjusted every two months to allow the animals to gain 8% of their initial free-feeding weights, up to 500 g. Once the latter weight was reached, the rat was maintained at that weight for the remainder of the study. All rats were individually housed in hanging wire-mesh cages with ad lib access to water. They were fed their allotment of lab chow approximately 2 hours after their single daily operant sessions. The animal quarters were kept on a 12:12 light-dark cycle with light onset at 0800 CST.

Apparatus

Four identical Lafayette (Model 8000L), two-lever operant chambers measuring 22×28×22 cm were used. Each operant chamber was enclosed in a Lafayette sound-attenuating chamber. All programming and recording was accomplished with a Rockwell Aim-65 microprocessor and software system modified from Rayfield and Carney [55].

Drug Discrimination Training

As soon as the animals achieved 80% of their free-feeding weights, they were trained to lever press for 45 mg food BioServ food pellets by successive approximation (manual

shaping) on an FR1 schedule. All rats were trained and tested at the same time each day from 0900 to 1500. One-half the rats were shaped to press the right lever and the other half to press the left lever. Upon completion of this initial shaping procedure (i.e., when the rat obtained at least 50 food pellets in the 30-minute session), Phase 1 of drug discrimination training began. Completion of this phase required 12 days. All of these and subsequent sessions lasted 11 minutes. Twenty-one rats were trained by the following procedures to discriminate 32 mg/kg caffeine (free-base) from saline and ten were trained to discriminate 56 mg/kg theophylline (free-base) from saline. The latter caffeine and theophylline doses were judged to be approximately equivalent in terms of brain concentration levels of each drug (Carney and Christensen, Personal Communication).

Phase 1 training was designed to develp schedule-controlled lever responding first on the originally shaped lever after intraperitoneal (IP) injections of normal saline (No drug, lever-N) and then on the other lever after IP injections of the training drug (Drug lever-D). Twenty minutes prior to each training session, either the training drug or saline was injected and lever appropriate responding resulted in schedule-controlled delivery of food pellets. During Phase 1 training, six drug and six saline sessions were given with the reinforcement schedule for both Drug and Saline sessions progressively shifting from Fixed-Interval (FI) 1 to FI3, FR3, FR5, VR 5–10 and then to VR 5–15. This terminal variable ratio schedule was chosen because it produces a sufficient level of responding during the extinction test sessions (see below) to permit simultaneous assessment of both the test drug's stimulus properties and its effects on response rate.

Phase 2 training began immediately after the completion of Phase 1. During the first minute of each 11-minute training session, no reinforcement was available (extinction) and during the last 10 minutes of each session, reinforcement was available on the VR 5–15 schedule for injection-appropriate lever presses (i.e., N or D). A double-alternation sequence of training sessions under D or N injection/reinforcement conditions was used throughout Phase 2 training and for the remainder of the experiment. With Phase 2 training sessions given only five days a week, the weekly sequence repeated itself once every 5 weeks. All rats continued Phase 2 training until they reached a criterion of 8 out of 10 sessions with 70% or greater drug-appropriate responding during the initial 1-minute extinction period of each training session. Sessions to criterion was designated as the number of sessions from the beginning of Phase 2 to the first session of the 10 days criterion sequence. After reaching criterion, all rats received additional training sessions until an asymptotic discrimination performance criterion was met, i.e., 10 sessions with 70% or greater drug discrimination and within ±10% of the mean for those 10 sessions.

Drug Discrimination Tests

Drug discrimination test sessions lasted 2 minutes and no reinforcement was available. If criterion level performance was maintained, test sessions were given on Tuesdays and Fridays. The latter schedule was superimposed on the double-alternation training session sequence which repeated itself every 5 weeks. Every test drug/dose combination was assessed after both D and N training days. Both caffeine- and theophylline-trained rats were first tested for generalization to the training drug and to other xanthines (see [42]) and then for generalization to or blockade by L-PIA, CDP, and pentobarbital (PBL). Finally, a small sample of caffeine-trained rats provided limited testing of papaverine's (PPV) generalization to the caffeine cue.

Drugs and Drug Discrimination

Caffeine and theophylline were obtained from Eastman Kodak Chemicals. The chlordiazepoxide hydrochloride was generously donated by Hoffman-LaRoche, Inc. The sodium pentobarbital (Nembutal) was purchased from Abbott Labs. The papaverine (6,7-dimethoxy-1-verarylisiquinoline) hydrochloride was obtained from Sigma Chemical Company. Only the caffeine and theophylline doses refer to base. All drugs were mixed with normal saline such that 1.0 ml contained the training or test dose for a 1 kg rat. The IP injections of all training or test drugs were given 20 minutes prior to the sessions. On blocking test sessions, an IP injection of one of the following drug/dose combinations was administered 2 minutes before the injection of the training-drug dose: (a) 0.01, 0.05, or 0.1 mg/kg L-PIA; (b) 1.0, 5.0, or 10.0 mg/kg CDP; and (c) 2.5 or 5.0 mg/kg PBL. Several doses of L-PIA (0.01–0.1 mg/kg) and CDP (1.0–10.0 mg/kg) also were tested for generalization to both xanthine cues. Finally, several doses of PBL (2.5–5.0 mg/kg) and PPV (0.5–12.0 mg/kg) were tested respectively for blocking of and generalization to the caffeine cue.

Data Analysis

On test days, both % drug-appropriate responding (D lever/(N+D levers) × 100) and total response rate (N+D levers) were recorded. Analysis of variance tests (ANOVAs) were run on both measures on generalization and/or blocking tests for a given drug and a given training cue. Two-way ANOVAs were performed on the L-PIA and CDP drug data (drug dose by presence or absence of the xanthine training dose). One-way ANOVAs were performed on the pentobarbital blocking data (within subjects across dose levels). The data for generalization tests included a saline test day; those for blocking tests included a training drug test day. Post-hoc comparisons among doses were made with Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Theophylline- and caffeine-trained rats achieved criterion drug-lever responding in 13.7 and 14.9 sessions (means) respectively after the completion of Phase 1 training.

CAFFEINE TRAINING CUE

Table 1 shows the % caffeine-lever responses, the percent of rats at or exceeding criterion drug discrimination (70% or greater drug-appropriate responding), and overall response rate for: (a) training drug conditions; (b) generalization and blocking tests with L-PIA; (c) generalization and blocking tests with CDP; and (d) blocking tests with pentobarbital. The 32 mg/kg caffeine training condition produced significantly higher % caffeine-lever responses (paired $t(10)=20.04$, $p<0.001$) and significantly lower response rates (paired $t(10)=2.91$, $p<0.02$) than did the saline condition. This response rate decrease is comparable to that reported in other studies [11,42].

L-PIA Tests

As seen in the second section of Table 1, L-PIA did not generalize to the caffeine cue, $F(2,44)=2.79$, n.s., not signifi-

cantly differing from saline at any dose tested. Further, no dose of L-PIA blocked the 32 mg/kg caffeine training cue, $F(2,44)<1$, i.e., no L-PIA dose plus 32 mg/kg caffeine differed significantly from the caffeine training dose alone. The overall response rate measure recorded during the same generalization and blocking tests indicated a biphasic effect for L-PIA's influence on responding, $F(3,66)=28.35$, $p<0.01$, relative to saline, with increases in responding at 0.01 mg/kg ($p<0.05$) and decreases in responding at 0.05 and 0.1 mg/kg doses (p's<0.01). The response rate measure indicated an interaction effect when caffeine and the several L-PIA doses were combined, $F(3,33)=8.05$, $p<0.01$. No dose of L-PIA in combination with caffeine differed significantly from caffeine alone. The 32 mg/kg caffeine dose in combination with 0.01 mg/kg L-PIA respectively lowered response rates relative to saline alone ($p<0.01$) and to the 0.01 mg/kg L-PIA dose alone ($p<0.01$). At higher L-PIA doses (0.05 and 0.1 mg/kg), the addition of 32 mg/kg caffeine increased response rate relative to each of the respective L-PIA doses alone ($p<0.05$ and $p<0.01$, respectively).

In summary, L-PIA neither generalized to nor blocked caffeine discrimination when combined with the caffeine training dose. L-PIA alone yielded a biphasic, dose-dependent effect on response rate, with increases at the lowest and decreases at the highest doses. When combined with the training dose of caffeine, all of L-PIA's dose-related effects on response rate were blocked. Thus, the failure of L-PIA to block the caffeine cue cannot be attributed to the use of L-PIA doses which had no behavioral effects or which did not interact with caffeine.

CDP Tests

These data can be seen in the middle portion of Table 1. No dose of CDP produced caffeine-lever responding that was significantly different from saline (overall $F(3,66)=1.21$, n.s.). However, CDP did produce a dose-dependent decrease in caffeine-lever responding when combined with the 32 mg/kg training dose, $F(3,66)=11.64$, $p<0.01$. When compared to the training dose alone, all doses of CDP in combination with caffeine produced significant decreases in % caffeine-lever responding (p's<0.01). All combinations of CDP and caffeine still produced levels of caffeine-lever responding which were significantly higher than those following saline injections. However, caffeine-appropriate responses produced by the 10 mg/kg CDP dose in combination with caffeine were not significantly different from those produced by 10 mg/kg CDP without caffeine. Caffeine in combination with 1.0 and 5.0 mg/kg CDP did yield significantly more drug lever responses than the same CDP doses given alone (all p's<0.01).

CDP alone produced dose-dependent changes in response rate during the test sessions, $F(3,66)=24.39$, $p<0.01$, similar to those produced by L-PIA. The 1 mg/kg CDP dose yielded higher response rates than saline or any other CDP dose (p's<0.01), while the 10 mg/kg CDP dose decreases response rate relative to saline and the other CDP doses. The caffeine plus 10 mg/kg CDP combination produced lower response rates than caffeine alone or in combination with the 1 or 5 mg/kg CDP doses (p's<0.01). When each caffeine-CDP dose combination was compared with the comparable dose of CDP alone, the combination yielded significantly lower response rates (p's<0.01) except at the 10 mg/kg dose.

In summary, while CDP alone had no caffeine-cue properties, CDP did block the caffeine cue in a dose-dependent fashion. CDP had a dose-dependent, biphasic effect on response rate when given alone, and the rate-increasing effects of lower doses of CDP were blocked by the training dose of caffeine.

TABLE 1

TESTS FOR GENERALIZATION TO AND BLOCKADE OF THE 32 mg/kg CAFFEINE CUE WITH L-PHENYLISOPROPYLADENOSINE (L-PIA), CHLORDIAZEPOXIDE (CDP), AND PENTOBARBITAL (PBL) (MEAN ± S.E.)

Drug Condition/ Drug Dose	% Caffeine Lever Responses	% Subjects with ≥70% Caff Rs/ Significance*	Total Responses
Training Drugs: n=12			
32 mg/kg Caffeine	81.6 ± 3.3	100/—	32.2 ± 5.1
Saline	8.8 ± 2.3	0/.001	63.9 ± 11.6
L-PIA Alone: n=12			
0.01 mg/kg L-PIA	7.2 ± 2.0	0/.001	95.4 ± 11.3
0.05	19.0 ± 6.5	0/.001	13.9 ± 6.7
0.1	(0)	0/—	0.2 ± 0.1†
32 mg/kg Caffeine + L-PIA: n=12			
0.01 mg/kg L-PIA	74.9 ± 7.4	75/n.s.	56.2 ± 11.3
0.05	77.8 ± 3.8	75/n.s.	40.4 ± 9.2
0.10	72.2 ± 7.4	75/n.s.	38.9 ± 9.1
CDP Alone: n=12			
1.0 mg/kg CDP	9.1 ± 4.2	0/.001	90.4 ± 11.1
5.0	8.7 ± 3.0	0/.001	62.5 ± 10.2
10.0	21.8 ± 6.9	0/.001	11.2 ± 3.5
32 mg/kg Caffeine + CDP: n=12			
1.0	59.1 ± 8.7	42/.01	45.8 ± 5.7
5.0	50.8 ± 8.3	17/.001	31.3 ± 8.0
10.0	34.0 ± 6.1	17/.001	7.6 ± 3.5
32 mg/kg Caffeine + PBL: n=6			
0.0 mg/kg PBL	88.3 ± 4.1	100/—	33.5 ± 7.3
2.5	68.5 ± 4.0	67/n.s.	22.0 ± 8.0
5.0	76.4 ± 7.6	67/n.s.	14.8 ± 3.5

*% Subjects with % caffeine-lever responses of 70 or higher. Probabilities associated with McNemar Change Test (Chi square with $df=1$), comparing % subjects with ≥70% caffeine lever responses on Generalization or Blocking Tests relative to that for the Training Drug Dose (n.s.=non-significant).

†Only one rat responded.

Pentobarbital Tests

A subgroup of six rats (non-systematically selected) were tested for blockade of the 32 mg/kg caffeine cue by pentobarbital (PBL). These data can be seen in the lower portion of Table 1. While there was a significant difference across doses, $F(2,10)=5.19$, $p<0.05$, a unidirectional dose-

TABLE 2

CAFFEINE CUE GENERALIZATION TESTS FOR PAPAVERINE (PPV; n=3) (MEAN ± S.E.)

Drug/Dose (mg/kg)	% Caffeine Lever Responses	Saline Comparison (% Caff Rs)*	Training Drug Comparisons (% Caff Rs)*	Total Responses
32 mg/kg Caffeine	75.0 ± 5.6	$p<0.01$	—	32.8 ± 9.9
Saline	14.7 ± 6.5	—	$p<0.01$	52.5 ± 23.7
0.5 mg/kg PPV	4.2 ± 1.9	n.s.	$p<0.01$	38.5 ± 13.6
1.5 mg/kg PPV	1.5 ± 1.1	n.s.	$p<0.01$	34.2 ± 12.3
5.0 mg/kg PPV	25.6 ± 12.6	n.s.	$p<0.05$	29.7 ± 10.4
7.5 mg/kg PPV	23.5 ± 11.3	n.s.	$p<0.01$	31.7 ± 14.9
9.0 mg/kg PPV	73.3 ± 10.5	$p<0.01$	n.s.	18.2 ± 5.9
12.0 mg/kg PPV	47.8 ± 21.9	n.s.	n.s.	15.8 ± 12.2

*Probabilities associated with comparisons of % caffeine-lever responses between papaverine test doses and either saline or the training dose of caffeine.

dependent effect was not apparent. The caffeine alone condition produced significantly higher caffeine-lever responses that the caffeine + 2.5 mg/kg PBL ($p<0.05$), but was not different from the caffeine + 5.0 mg/kg PBL condition. Further, the lowest level of drug responding was only just below criterion level. While dose-dependent declines in response rate were apparent, no significant differences were found.

In summary, PBL slightly attenuated but did not block the caffeine cue. These data support the contention that the decrease in caffeine responding found with higher doses of CDP likely was not due to any general sedative property of CDP.

Papaverine Tests

A partial test of the hypothesis that the xanthine cue is based on its capacity to inhibit phosphodiesterase was provided by PPV generalization tests in a subgroup of three caffeine-trained rats. These test data are presented in Table 2. Although the sample is small, papaverine produced a clear dose-related generalization to the caffeine cue, $F(7,14)=6.94$, $p<0.01$, and a nonsignificant but dose-related decrease in total operant responding. The % caffeine-appropriate responses found after the two highest PPV doses was significantly higher than that seen after the two lowest PPV doses (all p's<0.05) and was not significantly different from drug-lever responding found with the caffeine training dose (32 mg/kg). However, only the 9 mg/kg papaverine dose produced significantly more caffeine-lever responses than that found with saline ($p<0.05$).

In summary, PPV appeared to generalize to the caffeine cue in a dose-related manner. This finding provides preliminary support for the contention that the basis for the caffeine cue may involve caffeine's effects on c-AMP mechanisms.

THEOPHYLLINE TRAINING CUE

Table 3 shows the mean percent theophylline-lever responding, % of rats responding on the theophylline lever at criterion levels (70% or greater), and the response rate during the test session for: (a) training drug conditions; (b) L-PIA alone or in combination with the 56 mg/kg theophylline training dose; and (c) CDP alone or in combination with theophylline. The theophylline training dose yielded significantly higher % theophylline-lever responding (paired $t(6)=15.42$, $p<0.001$) and significantly lower response rates (paired $t(6)=3.15$, $p<0.02$) than did the saline condition.

L-PIA Tests

These data are seen in the second portion of Table 3. Whether alone or in combination with theophylline, no significant differences in theophylline responding were found among the two L-PIA doses. No dose of L-PIA alone differed significantly from the saline condition. Further, at both L-PIA doses, the combination of L-PIA plus theophylline training dose resulted in significantly greater drug-lever responding than the comparable L-PIA dose alone (p's<0.01).

L-PIA alone produced a dose-dependent decline in response rate, $F(2,28)=12.42$, $p<0.01$, with the 0.05 mg/kg dose yielding lower response rates than saline ($p<0.05$) and the 0.1 mg/kg dose producing smaller response rates than either saline alone ($p<0.01$) or the 0.05 mg/kg L-PIA dose ($p<0.05$). In combination with the 56 mg/kg theophylline training dose, no significant differences in response rate were detected among L-PIA doses. In addition, neither combination differed from theophylline alone. All of the latter response rates were relatively low. Finally, no significant differences in response rate were found between comparable L-PIA doses given alone or in combination with theophylline.

In summary, L-PIA neither generalized to nor blocked the theophylline cue. Finally, the dose-dependent decrease in response rate seen with L-PIA alone was absent when L-PIA was given in combination with the theophylline training dose.

CDP Tests

The CDP-alone tests produced significant differences among doses, $F(2,28)=4.20$, $p<0.05$, in % theophylline-lever responses, with the 10 mg/kg dose producing higher values

than either saline alone or the 5.0 mg/kg dose. However, both the 5.0 and 10 mg/kg doses of CDP were significantly different from the theophylline training dose ($p<0.001$). In combination with the theophylline training dose, significant differences were found among CDP doses for % theophylline-lever responding, $F(2,28)=9.06$, $p<0.01$. The two CDP-theophylline combinations did not significantly differ from one another in % drug-lever responses. Both CDP-theophylline combinations produced significantly lower % drug-lever responses than that produced by the theophylline training dose alone (p's<0.01), but significantly higher % drug-lever responses than the respective CDP dose alone ($p<0.05$).

A dose-dependent decrease in response rate was found in the tests with CDP alone, $F(2,28)=17.34$, $p<0.01$, with the 10 mg/kg dose yielding lower rates than either saline or the 5 mg/kg dose ($p<0.01$ and $p<0.05$, respectively). When CDP doses in combination with the theophylline training dose were examined, no significant overall difference in response rate was found ($F<1$). However, the 5.0 mg/kg CDP dose in combination with theophylline produced significantly lower rates than that dose of CDP alone, $F(1,21)=5.78$, $p<0.05$.

In summary, CDP alone did not have theophylline cue properties but did produce dose-dependent decreases in operant responses. In combination with the theophylline training dose, CDP attenuated the theophylline cue and appeared to additively interact with theophylline in affecting response rate. CDP's blockade of the theophylline cue was not as complete as was the case for the caffeine cue.

TABLE 3

TESTS FOR GENERALIZATION TO AND BLOCKADE OF THE 56 mg/kg THEOPHYLLINE CUE WITH L-PHENYLISOPROPYLADENOSINE (L-PIA), AND CHLORDIAZEPOXIDE (CDP) (MEAN ± S.E.)

Drug Condition/ Drug Dose	% Theophylline Lever Responses	% Subjects with ≥70% Theo Rs/ Significance*	Total Responses
Training Drugs: n=8			
32 mg/kg Theophylline	89.9 ± 4.6	100/—	16.5 ± 4.7
Saline	4.8 ± 2.0	0/.01	68.4 ± 13.4
L-PIA Alone: n=8			
0.05 mg/kg L-PIA	0.9 ± 0.8	0/.01	32.5 ± 13.9
0.01	7.6 ± 6.2	0/.01	2.9 ± 1.2
56 mg/kg Theophylline + L-PIA: n=8			
0.05 mg/kg L-PIA	81.1 ± 7.1	75/n.s.	24.8 ± 7.5
0.10	81.7 ± 8.2	87/n.s.	8.0 ± 5.4
CDP Alone: n=8			
5.0 mg/kg CDP	4.9 ± 3.1	0/.01	46.6 ± 6.8
10.0	27.6 ± 9.6	0/.01	10.4 ± 4.1
56 mg/kg Theophylline + CDP: n=8			
5.0 mg/kg CDP	63.5 ± 11.7	50/.05	19.5 ± 7.0
10.0	52.4 ± 11.1	37/.025	6.1 ± 2.2

*% Subjects with % theophylline-lever responses of 70 or higher. Probabilities associated with McNemar Change Test (Chi square with $df=2$), comparing % subjects with ≥70% theophylline lever responses on Generalization or Blocking Tests relative to that for the Training Drug Dose (n.s.=non-significant).

EXPERIMENT 2

RATIONALE

The blockade or attenuation of the xanthine cues by CDP appears relatively specific in that neither L-PIA (clearly a potent behavioral depressant at higher doses) nor pentobarbital produced similar changes in xanthine discrimination when xanthine training doses were used. That L-PIA did not alter the caffeine cue is particularly interesting, since there are two reports in the literature [18,62] indicating that caffeine blocks or attenuates the L-PIA cue (again two-lever food-rewarded drug discrimination in rats). These data raise the question of whether caffeine would block a CDP discriminative cue. Further, the finding that BDZs inhibit adenosine uptake and thus potentiate adenosine effects [1, 2, 67] raises the question of whether L-PIA would generalize to such a cue. The second experiment addressed these issues. While CDP is not one of the more potent BDZ agonists, a recent report shows that the CDP cue is blocked by the specific BDZ receptor blocker RO 15-1788 [32].

METHOD

Eight male Sprague-Dawley rats (same age, housing conditions, and food deprivation conditions as in Experiment 1) were trained to discriminate a 5.0 mg/kg dose of CDP from saline. All injection and training and testing conditions were the same as those used in Experiment 1.

RESULTS AND DISCUSSION

Table 4 presents the data for tests with the CDP training dose and saline, for the L-PIA generalization test, and for the caffeine blocking tests. The 5.0 mg/kg CDP training dose produced significantly higher % CDP-lever responses (paired $t(6)=12.21$, $p<0.001$) than did the saline condition but was not different from saline on the response rate measure (paired $t<1.0$).

L-PIA did not generalize to the CDP cue as indicated by the fact that no L-PIA dose produced % CDP-lever responding which was significantly different from saline ($F<1$). L-PIA did produce dose-dependent decreases in test-session response rates, $F(3,21)=2.28$, $p<0.01$), with the 0.1 mg/kg and the 0.05 mg/kg doses producing signficantly lower rates than 0.01 mg/kg or saline (p's<0.01).

Caffeine failed to block the 5 mg/kg CDP cue at any dose tested ($F<1$). However, in combination with the CDP training dose, caffeine did produce a dose-dependent decrease in test-session response rate, $F(3,15)=12.68$, $p<0.01$, with the 56 mg/kg dose producing lower rates than all other dose conditions ($p<0.01$). The 32 mg/kg dose yielded lower rates than did the 10 mg/kg dose ($p<0.05$) but not significantly lower than saline.

In summary, both L-PIA and caffeine significantly increased test-session response rates in CDP-trained rats but did not appear to interact with the CDP cue.

GENERAL DISCUSSION

The present data and other recent studies suggest a rather complicated state of affairs relative to the discriminative properties of xanthines, BDZs, and L-PIA and the interactions between these drugs. At least two recent reports indicate that low doses of caffeine (2.5–5 mg/kg) are sufficient to completely block the discriminative cue produced by 0.032–0.08 mg/kg of L-PIA [18,62]. It should be noted that this L-PIA cue likely was dependent on central rather than peripheral actions (e.g., hypotensive) of this adenosine analog, since the L-PIA cue was not blocked by 8-p-sulfophenyl-theophylline (a peripherally-acting xanthine; [13]). Such data support the hypothesis that the xanthine cue, like other behavioral effects of methylxanthines [30, 36, 37], may depend on some direct interactions with adenosine receptor mechanisms. For example, if the cue in caffeine-trained rats depended on some type of competitive blockade of the A1 adenosine receptor (which stereospecifically binds L-PIA), then L-PIA should block the caffeine cue and caffeine should block the L-PIA cue. The latter effect (caffeine blockade of the L-PIA cue) occurs. However, the present data show that L-PIA in the doses tested (0.01–0.1 mg/kg) does not block xanthine discrimination when combined with the training doses of either caffeine or theophylline. Others also report instances in which methylxanthine effects are not reversed by adenosine agonists. For example, IBMX induced accelerated norepinephrine turnover and enhanced NE enzyme activity, effects not altered by 2-chloradenosine [29].

The xanthine training doses used in the present study did significantly reduce overall response rate. Caffeine (32 mg/kg) reduced responding by 50% and theophylline (56 mg/kg) by 75%. These results replicate earlier results [11,42]. It is possible that the training doses of xanthines were too high to permit any blockade of the xanthine cue by the highest dose of L-PIA tested. While this possibility cannot be completely ruled out, other aspects of the data make it seem unlikely. Some have suggested that certain adenosine receptor sites may have "super-high" affinity for L-PIA and that these sites are less affected by the methylxanthine antagonists [30]. When higher doses of methylxanthines and of L-PIA are tested, lower affinity adenosine sites are blocked by methylxanthines but not the "super-high" affinity sites [35,36]. The present data indicate that the highest L-PIA test dose (0.1 mg/kg) clearly was pharmacologically effective in that it reduced operant response rates by over 90% in both caffeine- and theophylline-trained rats. Further, L-PIA in combination with the training doses of either caffeine or theophylline produced interactive effects on the operant response rate measure. Others [13,18] have reported similar findings, suggesting that, at least for operant responding, competitive interactions between methylxanthines and L-PIA do occur, probably at the A1 receptor. This set of data suggest that: (a) the primary mechanism for xanthine effects on operant behavior may be the blockade by methylxanthines of the adenosine A1-type receptors; and (b) the mechanism for the methylxanthine cue does not depend on any direct interaction with the adenosine A1 receptor.

In examining possible xanthine/BDZ interactions as a basis for the xanthine cue, a similar analysis is appropriate. If the xanthine cue in caffeine-trained rats depended on competitive blockade of BDZ receptor mechanisms *per se*, then one would expect mutual antagonism of either cue by the other drug. However, an asymmetrical relationship was found, i.e., CDP blocks the caffeine cue but caffeine does not block the CDP cue. This result is not too surprising, since methylxanthines have a relatively low potency in competitively displacing diazepam from BDZ receptor binding sites [7,41]. One of the principal actions of agonists for the BDZ receptor is to potentiate GABAergic transmission [7]. Perhaps caffeine acts either as an inverse agonist at the BDZ receptor with a much lower affinity than CDP for such receptors, or as a depressant of GABAergic transmission. Such a set of conditions might explain why CDP blocks the caffeine cue but caffeine does not block the CDP cue. Of interest here are the findings that other more potent inverse agonists for the benzodiazepine receptor, the beta carbolines, do block the CDP cue [32]. If the basis for the caffeine cue were its action at the BDZ receptor either as a blocker or as an inverse agonist, then one would expect cross-generalization between a methylxanthine discriminative cue and BDZ blocker such as RO 15-1788 and/or an inverse agonist like beta-carboline. Unfortunately, little data is available concerning the latter situation or concerning caffeine's effects on GABA mechanisms. Support for the caffeine/GABA hypothesis would be a positive generalization test for bicuculline (a GABA receptor blocker) or picrotoxin (an inhibitor of GABAergic activity acting at the chloride ionophore) in caffeine-trained rats. In this regard it will be recalled that in the present study, pentobarbital, which potentiates GABA effects and enhances the affinity of BDZs (presumably by acting at the chloride channel; [7]), attenuated but did not block the caffeine cue. Thus, the possibility that some BDZ/GABA mechanism provides a link necessary for the production of the caffeine cue remains open.

TABLE 4

TESTS FOR GENERALIZATION TO AND/OR BLOCKADE OF THE 5.0 mg/kg CHLORDIAZEPOXIDE CUE WITH L-PIA AND CAFFEINE (MEAN ± S.E.)

Drug Condition/ Drug Dose	% CDP Lever Responses	% Subjects with ≥70% CDP Rs/ Significance*	Total Responses
Training Drugs: n=8			
5.0 mg/kg CDP	84.6 ± 6.6	100/—	63.1 ± 6.0
Saline	3.6 ± 7.8	0/.01	61.1 ± 6.9
L-PIA Alone, n=8			
0.01 mg/kg	4.8 ± 3.3	0/.01	54.8 ± 7.2
0.05	3.7 ± 3.3	0/.01	30.5 ± 7.2
0.1	(6.0)	0/—	(2.0)†
5.0 mg/kg CDP + Caffeine: n=6			
0.0 mg/kg	92.1 ± 2.7	100/—	60.8 ± 12.1
10.0	92.2 ± 7.6	100/n.s.	63.5 ± 7.6
32.0	82.9 ± 7.3	83/n.s.	51.8 ± 7.6
56.0	81.3 ± 3.7	83/n.s.	27.5 ± 3.8

*% Subjects with % CDP-lever responses of 70 or higher. Probabilities associated with McNemar Change Test (Chi square with $df=1$), comparing % subjects with ≥70% CDP lever responses on Generalization or Blocking Tests relative to that for the Training Drug Dose (n.s.=non-significant).

†Only one rat responded.

Like L-PIA, CDP does interact with methylxanthines in affecting operant response rate. The highest CDP dose tested reduced response rates by 80–90%. In caffeine-trained animals, the addition of the caffeine training dose produced a reduction of response rate at each CDP dose tested relative to that seen with CDP alone. In CDP-trained animals, caffeine produced a dose-dependent decrease in response rate when administered with the CDP training dose. It recently has been found [1, 48, 67] that BDZs can be reasonably effective inhibitors of adenosine reuptake, and thus act by promoting adenosine agonist activity. Indeed, there is evidence that therapeutic efficacy of BDZ compounds is correlated with their potency as adenosine uptake inhibitors [67]. There is also some indication (D. Spencer, Personal Communication) that diazepam may generalize to an L-PIA training cue. These findings suggest that a common mechanism may exist for the CDP and L-PIA interaction with xanthines altering response rate, one involving enhanced agonist activity at the A1 receptor. However, the fact that L-PIA does not generalize to the CDP cue and that the CDP cue is not blocked by caffeine clearly indicates that the CDP cue itself does not depend on adenosine mechanisms.

Another candidate mechanism for the xanthine cue (considered earlier) is the xanthine inhibition of the nucleotide enzyme, phosphodiesterase, which converts c-AMP to the inactive form, 5'-AMP [15]. Relatively high concentrations of caffeine are necessary to produce physiologically significant levels of inhibition [59]. Such inhibition results in accumulation of c-AMP and in all of the resultant consequences of c-AMP itself. It will be recalled that agonist activity at the adenosine A1-type receptor resulted in inhibition of adenylate cyclase [39,63]. Methylxanthine blockade of the A1 receptor also should facilitate accumulation of c-AMP, although opposite effects may occur depending on the receptor type and/or brain site (see [27]). The calmodulin-Ca^{++} complex (CAM) also influences c-AMP activity [16]. Membrane-bound CAM stimulates adenylate cyclase activity and c-AMP production and thus plays a critical role in initiating postsynaptic membrane activity [23]. Diazepam has been found to block actions of calmodulin [24]. If the caffeine cue depended on c-AMP stimulation and/or the consequences of such stimulation, then a benzodiazepine (e.g., CDP) acting to block c-AMP production might counteract the mechanism necessary for the generation of the caffeine cue. Also of interest in this regard is the finding that CDP and high doses of theophylline have opposite effects on CA^{++} concentrations in synaptosomes [47].

A partial test of the hypothesis that the xanthine cue is based on its capacity to inhibit phosphodiesterase was provided by the caffeine-generalization tests with papaverine (PPV), a fairly potent phosphodiesterase inhibitor [25]. Although this sample was small, PPV produced a clear dose-related generalization to the caffeine cue. These data would appear to provide preliminary support for the contention that the basis for the caffeine cue may involve this drug's effects on c-AMP mechanisms. Unfortunately, PPV is not a specific phosphodiesterase inhibitor. It has been shown to inhibit adenosine uptake [2, 66, 67] and to competitively inhibit diazepam binding to brain membrane BDZ receptors [67]. Whether PPV's inhibition of adenosine uptake involves A1 adenosine receptor mechanisms is not known. However, other studies show that PPV and caffeine can have opposite effects. For example, PPV has previously been shown not to mimic caffeine's potentiation of rat rotational behavior [26] but rather may inhibit such behavior [28]. Further, PPV (probably via its ability to inhibit adenosine reuptake) potentiates adenosine-induced suppression of firing in rat corticospinal neurons, while caffeine blocks this adenosine action [50]. Yet, PPV's property of generalizing to the caffeine cue cannot rest on its ability to inhibit adenosine uptake and thus to potentiate adenosine effects, since neither L-PIA itself nor CDP (also an adenosine reuptake inhibitor) generalize to either methylxanthine cue. While the present PPV data are consistent with the hypothesis that the caffeine cue depends on some c-AMP-linked mechanism, the fact that caffeine is only a weak phosphodiesterase inhibitor clearly requires further research on the involvement of second messenger system effects as mediators for the xanthine cue. One specific direction of such research would be to test a phosphodiesterase inhibitor which does not alter adenosine levels or have adenosine-type effects (e.g., Rolipram; see [26,28]) in rats trained to discriminate caffeine from saline. Furthermore, at this stage one cannot completely dismiss the possibility that PPV mimics the caffeine cue by its capacity to interact with the BDZ-GABA receptor complex [67].

In summary, the present data support the hypothesis that the interaction of methylxanthines with CDP and L-PIA in affecting operant response rate likely involves a mechanism by which methylxanthines block the adenosine A1 receptor. On the contrary, adenosine receptor mechanisms do not appear to mediate the xanthine discriminative stimulus. Rather, the present data indicate that the xanthine cue may depend on some interaction of methylxanthines with the BDZ-GABA complex and/or c-AMP mechanisms. Many in the field conclude that all of the suggested mechanisms for one or more of the effects of methylxanthines fall short in explaining the multifaceted actions of such purines (see [26, 53, 59]). With respect to the discriminative cue properties of methylxanthines, it is conceivable that several related mechanisms mediate this cue. However, the apparent specificity of the cue compels one to continue the search for more unitary explanations.

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