Pharmacology Biochemistry & Behavior, Vol. 15, pp. 157–159, 1981. Printed in the U.S.A.

Caffeine-Induced Stimulus Control

J. C. WINTER

Department of Pharmacology and Therapeutics, School of Medicine
State University of New York, Buffalo, NY 14214

Received 6 March 1981

WINTER, J. C. *Caffeine-induced stimulus control.* PHARMAC. BIOCHEM. BEHAV. 15(2) 157–159, 1981.—Six rats were trained to discriminate the effects of caffeine (60 mg/kg, pretreatment time: 1 hour) and saline in a two-lever choice task using a fixed ratio 10 schedule of water reinforcement. Stimulus control was assumed to be present when 80% or more of the first ten responses were appropriate for the treatment condition on each of five consecutive days. The mean number of sessions prior to the onset of criterion performance was 22 (SE=3; range=11–32). In trained subjects, doses of caffeine of 30, 10, and 3 mg/kg were followed by a progressively smaller proportion of responses on the caffeine-appropriate lever. Stimulus control by caffeine began to diminish about four hours after administration and was completely absent after 24 hours. The caffeine cue generalized partially to *d*-amphetamine and completely to aminophylline. Neither pizotyline nor spiperone antagonized stimulus control induced by caffeine.

Aminophylline Caffeine Stimulus control

IT IS known that a wide range of psychoactive drugs can function as discriminative stimuli in the rat. A partial list includes ethanol, barbiturates, and other depressants, stimulants of the amphetamine type, cannabinols, opiates, and LSD-like hallucinogens [1]. Thus it is curious that caffeine, a drug which is self-administered each day by many millions of people, produces a mild withdrawal syndrome upon discontinuation, and is sometimes associated with anxiety in normal subjects and exacerbation of thought disorder in schizophrenics, should fail to establish stimulus control in the rat. Following unsuccessful attempts to train caffeine (50 mg/kg, IP) in a T-maze shock escape test, it was concluded that "caffeine is nondiscriminable" and "virtually inactive" [12,13]. The same laboratory subsequently reported that a dose of 125 mg/kg produced stimulus control [14]. In animals trained with nicotine [11], pentobarbital [8] and fentanyl [4], the administration of caffeine was followed by saline-appropriate responding. In addition, caffeine failed to antagonize the stimulus properties of ethanol [16] and of chloridazepoxide [3]. Rats trained with *d*-amphetamine yielded intermediate responding when tested with caffeine [9,17] and complete generalization to caffeine was observed in rats trained with the antidepressant drug, buprion [7]. The present investigation reexamines the discriminability of caffeine versus saline in a two lever response choice task.

METHOD

Animals

Six female Wistar strain rats were used in these experiments. They were housed in pairs in quarters exposed to a natural light cycle. Body weight was maintained at about 80% of normal by restriction of water intake. Rat chow was freely available in the home cage. Prior to these experiments, the rats had received neither drugs nor behavioral training.

Apparatus

A standard small animal test chamber (Coulbourn Instruments model E10-10) housed in a larger light-proof and sound-insulated box was used for all experiments. The chamber contained two levers mounted at opposite ends of one wall. Centered between the levers was a dipper which delivered 0.1 ml tap water.

Procedure

Training and testing procedures were similar to those described earlier [6]. After learning to drink from the dipper, subjects were trained to depress first one and then the other of the two levers. The number of responses required for each reinforcement was gradually increased from one to ten and all subsequent training and testing employed a fixed ratio ten (FR10) schedule of reinforcement. Discrimination training was then begun. Each ten-minute session followed 60 min after one of two treatments, caffeine (60 mg/kg; IP) or saline. During training sessions, every tenth response on the appropriate lever for the drug condition was reinforced. In a similar fashion, responses on the saline appropriate lever were reinforced following the injection of saline. For three subjects, the left lever was designated as caffeine-appropriate and for the remaining subjects, responses on the right lever were reinforced following caffeine. During discrimination training, caffeine and saline were administered according to a double alternation sequence, i.e., C, C, S, S. . . . The distribution of the first ten responses between the two levers was recorded each day. Caffeine-induced stimulus control was assumed to be present when, in five consecutive sessions, eight or more of the initial ten responses were on the appropriate lever.

To determine the degree of similarity of other treatments to the training dose of caffeine (60 mg/kg; 60 min before

testing), cross tests were conducted in which other drugs, a dose of caffeine other than the training dose, or the training dose at a time other than 60 min, were administered. Cross tests as well as tests of antagonism were conducted each Friday so long as previous performance in the same week did not fall below a criterion of 80% correct responding. During cross tests, no responses were reinforced and the cross test was terminated after the emission of ten responses or after ten minutes. Response distribution during cross tests was compared with the distribution in the immediately preceding caffeine and saline sessions (control sessions). Evaluation of the ability of drugs to antagonize the stimulus properties of caffeine was done in sessions similar to cross tests in that the session was terminated after ten responses and the results were compared with control sessions. If less than ten responses were emitted in ten minutes, the session was excluded from analysis.

All cross test and antagonism data were compared with control data by means of individual applications of Wilcoxon's signed ranks test (one tailed [5]). Differences were considered to be significant if they would be expected to arise by random sampling alone with a probability less than 0.025.

Drugs

Caffeine (Aldrich Chemical Co., Milwaukee, WI); *d*-amphetamine sulfate (K & K Laboratories, Plainview, NY), aminophylline (Sigma Chemical Co., St. Louis, MO), and pizotyline (Sandoz Pharmaceuticals, Hanover, NJ) were dissolved in 0.9% saline. Spiperone (Janssen Pharmaceuticals, New Brunswick, NJ) was dissolved in a minimal volume of glacial acetic acid and diluted with saline. All drugs were administered IP in a constant volume of 1 mg/kg b.wt. The doses of amphetamine refer to the salt.

RESULTS

Caffeine-induced stimulus control was observed in each of the six subjects trained. The mean number of sessions prior to the onset of criterion performance was 22 (SE=3; range=11–32). The dose-response relationship for caffeine in subjects trained with a dose of 60 mg/kg and a pretreatment time of 60 min is shown in Table 1. Doses of 10 and 30 mg/kg yielded intermediate results, i.e., a response distribution significantly different from that in either training condition. The time course for the caffeine cue was found to be quite prolonged (Table 2) but its effects were completely absent after 24 hours.

The results of cross tests with *d*-amphetamine and theophylline are shown in Table 3. Doses of 0.8 and 1.5 mg/kg of *d*-amphetamine yielded intermediate results and no dose of *d*-amphetamine substituted completely for caffeine. In contrast, aminophylline (10 mg/kg) was indistinguishable from caffeine. Attempts to block the stimulus effects of caffeine with pizotyline (3 and 10 mg/kg; 60 min before testing), a serotonergic antagonist, and with spiperone (0.05–0.2 mg/kg; 30 min before testing), a dopaminergic antagonist, were unsuccessful. The doses of pizotyline and of spiperone chosen for use have previously been shown to antagonize the discriminative stimulus properties of LSD [19], a presumed serotonergic agonist, and of *d*-amphetamine (Winter, unpublished), a presumed indirectly acting dopaminergic agonist, respectively.

TABLE 1

DOSE-RESPONSE RELATIONSHIP FOR CAFFEINE AS A DISCRIMINATIVE STIMULUS

Dose (mg/kg)	N*	% Caffeine choice (SE)
0†	6	1 (1)
3	6	13 (8)
10	6	32 (15)
30	6	63 (18)
60†	6	100 (1)

*Six animals tested at each dose; N designates the number which emitted ten responses during the test session.
†Training.

TABLE 2

TIME COURSE FOR CAFFEINE AS A DISCRIMINATIVE STIMULUS

Dose* (mg/kg)	Pretreatment Time (hrs)	% Caffeine choice (SE)
0	1†	5 (4)
0	5	10 (8)
60	1†	98 (1)
60	3	95 (3)
60	4	85 (11)
60	5	79 (12)
60	6	65 (18)
60	14	34 (20)
60	24	3 (2)

*Six animals tested for each condition.
†Training.

TABLE 3

EFFECTS OF *D*-AMPHETAMINE AND AMINOPHYLLINE IN RATS TRAINED WITH CAFFEINE AS A DISCRIMINATIVE STIMULUS

Test drug (pretreatment time)	Dose (mg/kg)	N*	% Caffeine choice (SE)
d-Amphetamine			
(15 min)	0.3	6	29 (5)
	0.8	6	54 (9)
	1.5	6	62 (11)
	3	2	15 (5)
Aminophylline			
(15 min)	3	6	65 (11)
	10	6	98 (14)
	30	6	59 (7)
	100	1	10

*Six animals tested at each dose; N designates the number which emitted ten responses during the test session.

DISCUSSION

The dose-response relationship for caffeine (Table 1) is unremarkable and the time course following the training dose (Table 2) is consistent with a rather long duration of action for caffeine. Six hours following a dose of 100 mg/kg (IP) in the rat, plasma levels decline by only about 20% [15].

The intermediate results obtained in cross-tests of *d*-amphetamine in caffeine-trained subjects are compatible with the results of others [9,17]. Together these studies suggest the presence of a common component in the respective stimulus properties of caffeine and *d*-amphetamine. That aminophylline (theophylline ethylenediamine) should mimic caffeine is not surprising in view of the known overlap between the pharmacological effects of the xanthines [15]. However, the decline in caffeine-appropriate responding at a dose of theophylline of 30 mg/kg suggests the intrusion of another stimulus peculiar to aminophylline and conceivably due to ethylenediamine. The failure of either pizotyline or spiperone to antagonize caffeine suggests that neither serotonergic nor dopaminergic factors play an essential role in the stimulus complex induced by the drug.

NOTE ADDED IN PROOF

A recent abstract by Carney and Christensen [2] described the training of rats given caffeine (32 mg/kg) by mouth. The stimulus generalized to theophylline as well as to caffeine-containing beverages. A complete description will be found in Modrow *et al.* [10].

REFERENCES

1. Barry, H., III and E. C. Krimmer. Pharmacology of discriminative stimuli. In: *Drug Discrimination and State Dependent Learning*, edited by B. T. Ho, D. W. Richards and D. L. Chute. New York: Academic Press, 1978, pp. 3–32.
2. Carney, J. M. and H. D. Christensen. Discriminative stimulus properties of caffeine: Studies using pure and natural products. *Pharmac. Biochem. Behav.* **13:** 313, 1980.
3. Colpaert, F. C. Discriminative stimulus properties of benzodiazepines and barbiturates. In: *Advances in Behavioral Biology*, Vol. 22, edited by H. Lal. New York: Plenum, 1977.
4. Colpaert, F. C., C. Niemegeers and P. Janssen. The narcotic cue: Evidence for the specificity of the stimulus properties of narcotic drugs. *Archs int. Pharmacodyn. Thér.* **218:** 268–276, 1975.
5. Goldstein, A. *Biostatistics*. New York-London: MacMillan, 1964.
6. Hirschhorn, I. D. and J. C. Winter. Mescaline and lysergic acid diethylamide (LSD) as discriminative stimuli. *Psychopharmacologia* **22:** 64–71, 1971.
7. Jones, C. N., J. L. Howard and S. T. McBennett. Stimulus properties of antidepressants in the rat. *Psychopharmacology* **67:** 111–118, 1980.
8. Krimmer, E. C. Drugs as discriminative stimuli, Ph.D. Dissertation, University of Pittsburgh, 1974.
9. Kuhn, D. M., J. P. Appel and I. Greenberg. An analysis of some discriminative properties of *d*-amphetamine. *Psychopharmacologia* **39:** 57–66, 1974.
10. Modrow, H. E., F. A. Holloway and J. M. Carney. Caffeine discrimination in the rat. *Pharmac. Biochem. Behav.*, in press.
11. Morrison, C. F. and J. A. Stephenson. Nicotine injections as the conditional stimulus in discrimination-learning. *Psychopharmacologia* **15:** 351–360, 1969.
12. Overton, D. A. State dependent learning produced by addicting drugs. *Psychopharmac. Bull.* **9:** 29–31, 1973.
13. Overton, D. A. State-dependent learning produced by addicting drugs. In: *Opiate Addiction: Origins and Treatment*, edited by S. Fisher and A. M. Freedman. Washington, DC: Winston, 1973, pp. 61–73.
14. Overton, D. A. and S. K. Batta. Relationship between abuse liability of drugs and their degree of discriminability in the rat. In: *Predicting Dependence Liability of Stimulant and Depressant Drugs*, edited by T. Thompson and K. Unna. Baltimore: University Park Press, 1977, pp. 125–135.
15. Rall, T. W. Central nervous system stimulants, the xanthines. In: *The Pharmacological Basis of Therapeutics*, edited by A. G. Gilman, L. Goodman and A. Gilman. New York: MacMillan, 1980, pp. 592–607.
16. Schechter, M. D. Effect of propranolol, *d*-amphetamine and caffeine on ethanol as a discriminative cue. *Eur. J. Pharmac.* **29:** 52–57, 1974.
17. Schechter, M. D. Caffeine potentiation of amphetamine: implications for hyperkinesis therapy. *Pharmac. Biochem. Behav.* **6:** 359–361, 1977.
18. Spindel, E., M. Arnold, B. Cusack and R. J. Wurtman. Effects of caffeine on anterior pituitary and thyroid function in the rat. *J. Pharmac. exp. Ther.* **214:** 58–62, 1980.
19. Winter, J. C. Stimulus properties of phenethylamine hallucinogens and LSD: The role of 5-hydroxytryptamine. *J. Pharmac. exp. Ther.* **204:** 416–423, 1978.