*Pharmacology Biochemistry and Behavior*, Vol. 45, pp. 987–990, 1993
Printed in the U.S.A. 

0091-3057/93 $6.00 + .00


BRIEF COMMUNICATION

# Effects of Caffeine and PD 116,600 on the Differential-Reinforcement-of-Low Rate 72-S (DRL 72-S) Schedule of Reinforcement

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Received 21 January 1992

MAREK, G. J., T. G. HEFFNER, J. B. RICHARDS, R. A. SHAUGHNESSY, A. A. LI AND L. S. SEIDEN. *Effects of caffeine and PD 116,600 on the differential-reinforcement-of-low rate 72-s (DRL 72-s) schedule of reinforcement.* PHARMACOL BIOCHEM BEHAV 45(4) 987–990, 1993.—Caffeine and PD 116,600 were found to decrease the reinforcement rate and increase the response rate in rats performing under a differential-reinforcement-of-low rate 72-s (DRL 72-s) schedule of reinforcement. In contrast, antidepressant drugs previously have been found to increase the reinforcement and decrease the response rate. Caffeine has been found to test similar to antidepressant drugs on at least one other behavioral screen, but caffeine does not possess clinical antidepressant properties. These results provide further support for the DRL 72-s schedule as a behavioral screen for antidepressant drugs.

Caffeine PD 116,600 Antidepressant drugs Behavioral screen DRL 72-s schedule
Adenosine antagonists Psychomotor stimulants

THE differential-reinforcement-of-low rate 72-s (DRL 72-s) schedule of reinforcement previously has been shown to be uniquely and characteristically affected by antidepressant treatments such as tricyclic antidepressants, 5-HT uptake inhibitors, monoamine oxidase inhibitors, atypical antidepressants such as trazodone, mianserin, and ritanserin as well as electroconvulsive shock. These treatments all increase the reinforcement rate and decrease the response rate of rats performing under a DRL 72-s schedule (5,8,10,11,21). Drugs from other pharmacological classes such as antihistamines, anticholinergics, amphetamines, alpha-adrenergic antagonists, benzodiazepines, barbiturates, alcohol, and antipsychotic drugs do not test as antidepressants (6–8,10,11,21,22) although there is some controversy concerning antipsychotic drugs testing as "false" positives (2,13). The purpose of the present report is to further test the specificity of the DRL 72-s schedule as a screen for antidepressant drugs by testing a drug, caffeine, that has not been used in the treatment of depression but which tests as an antidepressant on one behavioral model of depression (15). In addition, the present study also determined the effects of a selective adenosine receptor antagonist, PD 116,600 (8-cyclopentyl-theophylline), on the DRL 72-s schedule. PD 116,600 has been shown to have a high affinity for $A_1$ adenosine receptors in vitro (3) and to block the CNS effects of an $A_1$ adenosine agonist in vivo in rats (4). Unlike caffeine and theophylline, PD 116,600 does not produce stimulation of spontaneous locomotor activity in rats, but it does augment intracranial self-stimulation responding in rats (4). Because the DRL 72-s schedule can distinguish between antidepressant-like and psychomotor stimulant-like profiles, this test was used to critically evaluate the possible antidepressant properties of PD 116,600.

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## METHOD

### *Animals*

Subjects for this study were eight male Sprague-Dawley rats (Holtzman, Madison, WI), weighing about 350 g when the drug treatments began. They were housed in suspended wire cages (36 × 23 × 19 cm) with two rats occupying each cage. The colony room was maintained at a relatively constant temperature (21–23°C) and humidity (30–60%). Fluorescent lighting was automatically turned on at 06:00 h and turned off at 20:00 h. Teklad 4% Rat Diet was available continuously except during sessions in the operant chamber. Rats were allowed free access to water for a 20-min period following each session in the operant chamber. They were then water-deprived for the remaining 22.5 h (approximately) until the subsequent session.

### *Apparatus*

Eight Gerbrands model C operant conditioning chambers (21 × 23 × 19 cm) served to house the rats during the behavioral sessions. Each chamber was equipped with a white house light and remained on throughout the entire session. A lever that operated a microswitch was mounted on one wall 3.0 cm from the side and 2.5 cm above a grid floor and 6.5 cm from an access port for a dipper that held 0.025 ml water. A downward force equivalent to approximately 15 g (0.15 N) operated the lever, constituting a response. When a response fulfilled the schedule requirements, the dipper was lifted from a water trough to an opening in the floor of the access port for 4 s, constituting a reinforcer. Each experimental chamber was enclosed in a sound-attenuating chamber equipped with a fan to provide ventilation and a masking noise.

### *Training*

Each rat was initially trained under a concurrent fixed-ratio 1 (FR 1), fixed-time 60-s schedule for water reinforcement. This schedule provided reinforcement for each response in addition to making water available following each 60-s interval in which there had been no response. The few rats that did not spontaneously acquire lever-pressing behavior within three daily 30-min sessions under this schedule were trained by the experimenter using the method of successive approximations. The rats were first reinforced for facing the lever, then for coming into closer physical proximity with the lever; then for touching the lever; and then for pressing the lever. After all the rats had acquired lever-pressing behavior, they were placed under a DRL 18-s schedule for 2 weeks, and the requirement was then raised to a DRL 72-s schedule. Under this schedule, responses that occurred at least 72 s after the previous response were reinforced. Responses that occurred less than 72 s after the previous response were not reinforced and required an additional 72 s to pass without any responding for the reinforcer to be available following a response. Drug treatments were initiated after 8 weeks when the animals' performance on the DRL 72-s schedule had stabilized (see 7 for a more economical training schedule). Responding on the DRL 72-s schedule was considered stable when the standard error of the mean total response rate for each rat over five consecutive sessions was not greater than 10% of the corresponding mean. Experimental sessions lasted for 1 h and were conducted 6 days/week during light hours.

### *Drug Administration*

Caffeine and PD 116,600 were dissolved in 10% emulphur 30 min prior to injection by a) weighing drug into the vial, b) adding 2–3 drops of 95% EtOH to wet the drug, c) adding straight emulphur (allowing for 10% final volume), d) adding the final volume of distilled water, and e) sonicating until the drug was dissolved or well suspended. The drugs were administered IP 30 min prior to testing on Tuesdays and Fridays. Vehicle (10% emulphur) was administered Mondays and Thursdays, the day prior to drug injections. The doses used in studying caffeine were 0.1, 1.0, and 10.0 mg/kg. The doses used in studying the PD 116,600 compound were 0.1, 0.5, 1.0, 2.0, 4.0, and 10.0 mg/kg. The doses were administered in an ascending manner except for PD 116,600. The 0.5, 2.0, and 4.0 mg/kg PD 116,600 doses were given in an ascending manner after the 0.1, 1.0, and 10.0 mg/kg doses because a small nonsignificant increase in reinforcement rate was observed at the 0.1 and 1.0 mg/kg doses. Both drugs were supplied by Parke-Davis (Ann Arbor, MI).

### *Schedule Control and Data Analysis*

The experimental chambers were connected to a PDP-11/73 microcomputer. The schedule contingencies were programmed and reinforcements, responses, and sequential interresponse times were recorded using a Super SKED Software System (23). The number of reinforcements and responses per session at each drug dose were compared to control values and tested for statistically significant differences with a one-way repeated measures analysis of variance (ANOVA). The control values were the mean reinforcement and response rate average over several days: the 5 days (including the initial vehicle injection) immediately preceding the first dose and the day preceding each drug injection. Therefore, there were 5 control days plus the number of drug injections. The control reinforcement and response rates for the caffeine experiment were 9.3 ± 0.9 (mean ± SEM) and 130 ± 18, respectively. The control reinforcement and response rates for the PD116,600 experiment were 10.2 ± 1.8 and 147 ± 34, respectively. Multiple comparisons were performed by using the Dunnett test (24). Significance was accepted at the $p < 0.05$ level.

In addition, an IRT analysis of the caffeine data was performed. This IRT analysis compares the obtained IRT distribution of an individual rat to a corresponding negative exponential distribution. The corresponding negative exponential distribution fits the expected appearance of the obtained IRT distribution if the rat had randomly emitted the same number of responses over the same interval. The present IRT analysis is an extension of a previous method (16) in that three metrics are obtained that allow for quantifying the DRL IRT distributions with respect to the negative exponential. The peak area (PkA) and peak location (PkL) describe the pause component of the IRT distribution. The PkA is the proportion of IRT distributions in the peak not accounted for by the corresponding exponential. The PkL is the median IRT duration of the peak. The third metric, the burst ratio (BR), quantifies the initial bursting component of the IRT distribution. The BR is the total number of obtained IRT distributions in the burst component divided by the total number of IRT durations predicted to occur in the burst component on the basis of the corresponding negative exponential. Repeated measures ANOVA were performed on these three metrics and followed by multiple comparisons with the Dunnett test (24). The present IRT analysis will be described in greater detail elsewhere (17).

## RESULTS

Caffeine decreased the reinforcement rate [$F(7, 21) = 10.81$, $p < 0.001$, see Fig. 1] at 10 mg/kg ($p < 0.01$) and increased the response rate [$F(7, 21) = 6.93$, $p < 0.01$] at 10

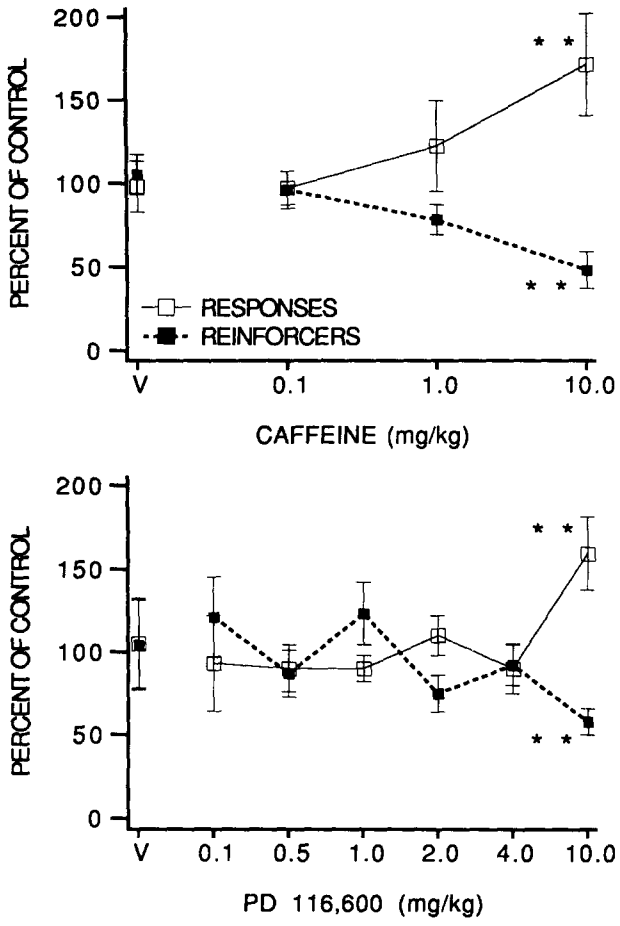


FIG. 1. Effect of caffeine and PD116,600 on reinforcement rate (■) and response rate (□) in rats responding under a DRL 72-s schedule. The points represent the mean ± SE for eight rats expressed as percent of control performance. Significantly different from control performance, $*p < 0.05$; $**p < 0.01$.

mg/kg ($p < 0.01$). PD 116,600 also decreased the reinforcement rate [$F(7, 42) = 5.42$, $p < 0.001$] at 10 mg/kg ($p < 0.05$) and increased the response rate [$F(7, 42) = 8.19$, $p < 0.001$] at 10 mg/kg ($p < 0.01$).

Caffeine decreased the PkA [$F(3, 21) = 8.41$, $p < 0.05$, see Fig. 2] and also decreased the PkL [$F(3, 21) = 11.96$, $p < 0.05$]. Caffeine did not significantly affect the BR [$F(3, 21) = 0.31$].

## DISCUSSION

Caffeine and PD 116,600 decreased the reinforcement rate and increased the response rate on the DRL 72-s schedule. These effects are similar to the effects of other psychomotor stimulants such as amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and methylphenidate on DRL behavior (6,12). Caffeine also decreased the PkA and PkL, without affecting the BR, just as does amphetamine (17). Thus, the adenosine antagonists result in similar effects on DRL behavior as do other classes of psychomotor stimulants.

While the adenosine antagonist PD 116,600 can be contrasted with caffeine and theophylline in that it does not stimulate spontaneous locomotor activity, the DRL 72-s schedule nonetheless reveals a profile for PD 116,600 that resembles that of psychomotor stimulants. This is in agreement with the ability of PD 116,600 to augment intracranial self-stimulation responding in rats (4), a profile seen with amphetamine and other stimulant drugs. The present data suggest that the DRL 72-s schedule provides a particularly sensitive test for distin-

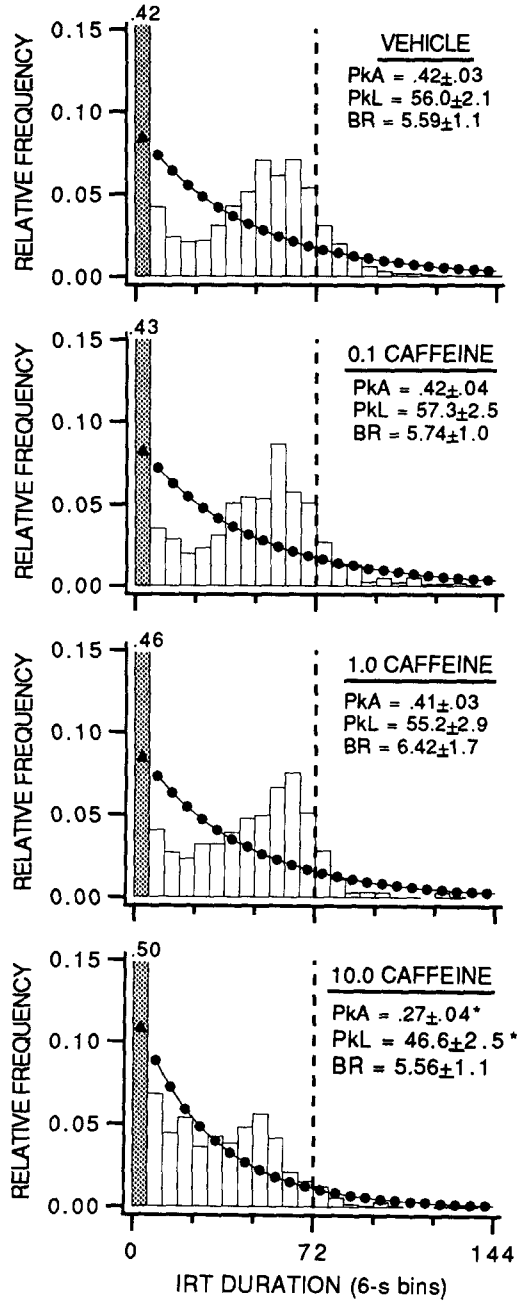


FIG. 2. Effect of caffeine on the IRT distribution. The four histograms show the effect of increasing doses of caffeine. The histograms show the relative frequency fo IRT distributio (bars) and corresponding negative expotential distribution (●) with burst IRTs excluded. Bursting is indicated by the shaded histogram bars. The dashed verticallines indicate the 72 s DRL criterion value. The histograms represent the average of four rats. The PkA, PkL and BR are displayed in the upper right hand corner of each histogram. Significantly different from control performance, $*p < 0.05$.

guishing between the antidepressant-like and stimulant-like effects of drugs, a distinction that has proven more difficult to make with use of other preclinical antidepressant tests.

Caffeine is a drug that is not known to exert clinical antidepressant effects. Yet caffeine does appear to test similar to antidepressant drugs on the forced swim test (14). In contrast, caffeine does not test similar to antidepressant drugs on the DRL 72-s schedule. Antidepressant drugs increase the reinforcement rate, decrease the response rate, and cause characteristic changes in the IRT distribution in rats performing under a DRL 72-s schedule (17,22). While amphetamines, caffeine, antihistamines, and anticholinergics all act similar to antidepressant drugs on the forced swim test (14), none of these drugs act similar to antidepressant drugs on the DRL schedule (6,9,10). Indeed, the antihistamine and anticholinergic effects of antidepressant drugs are felt to be responsible for many of their side effects (18,19). While there is some clinical lore regarding the clinical efficacy of amphetamines in treating affective disorders, double-blind placebo controlled trials do not support this belief (20). Thus, the DRL 72-s schedule, unlike the Porsolt test, is congruent with the lack of clinical efficacy of amphetamines, caffeine, antihistamines, and anticholinergics.

In contrast, the dopamine uptake inhibitors bupropion and nomifensine, which are antidepressants, both test similar to antidepressant drugs on the Porsolt test (1). Bupropion and nomifensine increase the response rate and decrease the reinforcement rate on DRL behavior (10,21) similar to other psychomotor stimulants and unlike other antidepressant drugs. The forced swim test is the most widely used behavioral antidepressant drug screen. The DRL 72-s schedule compares favorably to the forced swim test in that amphetamines, caffeine, antihistamines, and anticholinergics do not test similar to known antidepressant drugs. Nevertheless, at the present time no one behavioral antidepressant screen by itself is an adequate preclinical screen for antidepressant efficacy.

ACKNOWLEDGEMENTS

This research supported by U.S. Public Health Service Grant MH-11191 to L. Seiden who is also the recipient of a Research Scientist Award MH-RSA-10562. G. Marek was a Medical Scientist Training Program trainee supported by PHS Training Grant #5-T32-GM07281. The authors thank Barbara X. Knight for excellent assistance in the preparation of this manuscript.

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