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Relationship Between Caffeine Discrimination and Caffeine Plasma Levels[1]

HAROLD E. MODROW*, FRANK A. HOLLOWAY*, H. DIX CHRISTENSEN† AND JOHN M. CARNEY†

**Department of Psychiatry and Behavioral Sciences and †Department of Pharmacology*
University of Oklahoma Health Sciences Center
Oklahoma City, OK 73109

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MODROW, H. E., F. A. HOLLOWAY, H. D. CHRISTENSEN AND J. M. CARNEY. *Relationship between caffeine discrimination and caffeine plasma levels.* PHARMAC. BIOCHEM. BEHAV. 15(2) 323–325, 1981.—Rats, trained to discriminate 32 mg/kg caffeine from saline in a two-lever operant task, were tested for the presence of caffeine-appropriate lever responding at various intervals after the intraperitoneal injection of 32 mg/kg caffeine. Following completion of all behavioral tests, caffeine plasma levels were determined in the same animals at the same intervals after caffeine administration. After injection, both caffeine levels and caffeine-appropriate responding showed rapid increases followed by a differential decline.

Drug discrimination Operant behavior Caffeine Plasma level High pressure liquid chromatography

RECENTLY, caffeine has been shown to be capable of producing a xanthine-specific discriminable cue in rats [9]. This cue specificity was shown by the generalization of theophylline but not amphetamine, nicotine, or methylphenidate to the caffeine cue [9].

Although the drug cue used by animals in the drug-discrimination task is thought to depend on a central nervous system process [10], a correlation between the time parameters of caffeine's behavioral effects and changes in neural tissue concentration is unobtainable at this time. In part, this is because the anatomical substrate for caffeine's behavioral effects is not known. The most convenient internal measure is caffeine plasma level, on the assumption that plasma concentration will reflect brain levels. The purpose of the present study was to determine the relationship between behavioral caffeine discrimination and plasma caffeine levels.

METHOD

Animals

Six adult male Sprague-Dawley rats (Sasco, Inc.) were deprived to 80% of their free-feeding weight and maintained at that level throughout the study. All animals were housed in individual rack cages with ad lib water and were maintained on a 12:12 light:dark cycle throughout the study. The animals used in this study had previously been trained to discriminate 32 mg/kg caffeine from saline and had maintained criterion discrimination performance over the prior four months [9].

Apparatus

Four identical LeHigh-Valley two-lever operant chambers were used. Each animal was trained and tested in the same chamber throughout the study. All programming and recording was done by means of solid state equipment.

Procedure

Prior to training each day, animals were injected IP with either 32 mg/kg caffeine (free base; dissolved to a concentration of 10 mg/ml in physiological saline) or an equivalent volume of physiological saline. Twenty minutes after the injections, animals were placed in two-lever operant chambers and allowed to lever press for 45 mg Noyes food pellet reinforcements. One-half of the animals had previously been trained to respond on the left lever while in the saline state (right lever in the drug state) while the remaining animals were trained to respond to the right lever while in the saline state (left lever in the drug state). The twenty minute training sessions utilized a double alternation procedure with two caffeine training days following two saline training days (S,S,C,C,S,S, . . .).

Tests were administered every third day. Prior to testing each animal was injected with 32 mg/kg caffeine. According to a semirandom schedule, rats were tested 1, 5, 10, 20, 30, 60, 120, or 240 minutes after the caffeine injection. One-half of the tests at any point occurred after a caffeine training day while the other half occurred after a saline training day. This was done to avoid biasing responding to either of the two levers. A test session consisted of allowing the animal to

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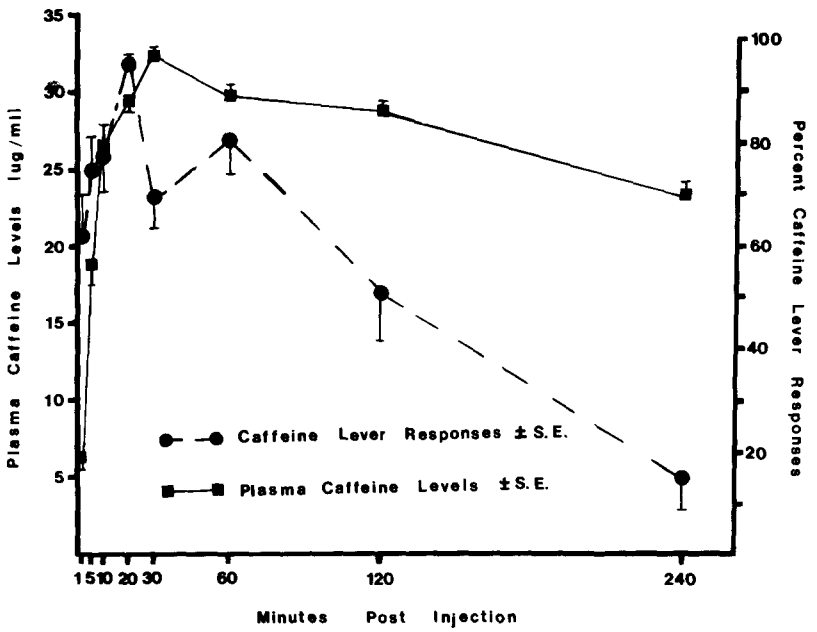

FIG. 1. Percent caffeine-appropriate lever responses compared to plasma caffeine levels for the period between 1 and 240 minutes post-injection.

respond on either lever until it had completed 30 presses on one lever. At that point, the animal was removed from the chamber and the test was concluded.

Following completion of all behavioral tests the caffeine plasma level was determined in all animals [1]. Blood samples (160 μl in heparinized microhematocrit tubes) were collected from the clipped tail vein 1, 5, 10, 20, 30, 60, 120, and 240 minutes after injection. The plasma was diluted 1:1 with the chromatographic organic solvent, consisting of 7% acetonitrite in 0.01 M sodium acetate (pH 4.0) containing β-hydroxyethyl-theophylline as an internal standard. After centrifugation to remove large proteins, the mixture was injected into a high pressure liquid chromotographic system (Waters ALC/GPC-244) containing a βBondapak C_{18} column. Quantification of caffeine concentrations was from the ratio of compound to internal standard peak heights.

RESULTS

The rats began responding on the caffeine lever within the first five minutes after drug injection. The percentage of caffeine-appropriate lever responses increased regularly during the 0 to 20 minute post-injection period. The percentage of drug-appropriate responses then decreased over the remaining tests. There was an apparent loss of discriminability of caffeine at 30 minutes. However, this was due to two animals responding on the saline lever. The use of a fixed ratio produces essentially quantal data in that animals tend to make all responses on one lever. Thus individual animals tend to either respond only on the saline or only on the caffeine-appropriate lever, but by summing across the population of animals, a graded effect curve is obtained. The average percentage of caffeine-appropriate lever responses for each test is shown in Fig. 1. As an ANOVA revealed significant differences between the different tests, $F(7,35)=2.689$, $p<0.05$, a Tukey post-hoc analysis was performed. It revealed the test 240 minutes post-injection differed significantly ($p<0.05$) from the tests 5, 10, 20, and 60 minutes post-injection. An ANOVA of the plasma caffeine levels (also seen in Fig. 1) revealed significant differences across time, $F(7,35)=16.234$, $p<0.001$. A Tukey post-hoc analysis showed that plasma caffeine level at the early tests (1 and 5 minutes post-injection) was significantly lower than that of the later tests (20, 30, 60, and 120 minutes post-injection).

Direct comparisons of at least two parameters for the time course of cue-appropriate behavior and drug level is permitted by nonparametric analyses. First, latency to peak was examined. No significant difference in latency to peak effect was found between the two measures. Next, the decline of behavioral and drug level measures was assessed by transforming values for both the behavioral and drug level data at the 4 hour point into percentage of peak response. The plasma levels 4 hours post-injection were 70.0% of their peak values while caffeine-appropriate lever responding was only 15.5% of the peak value. This difference was significant ($p<0.031$, Walsh test [12]). Thus, caffeine-appropriate lever responding declined more rapidly than did the plasma levels.

DISCUSSION

These results indicate that IP caffeine injections produce a discriminable cue with a rapid onset and a gradual decline. The behavioral data indicate that responding on the caffeine-appropriate lever begins within the first 5 minutes post-injection, reaching a peak at 20 minutes post-injection and declining over the later test intervals. These time parameters of onset and duration for the caffeine cue are similar to those for the amphetamine cue [6, 7, 13].

Both the peak and time course data for plasma caffeine at a comparably high caffeine dose were similar to those previously reported by Latini, *et al.* [8], Thithapandha *et al.* [15] and Spindel *et al.* [14]. Most notable in the present study is the finding that the plasma caffeine level on the declining

limb of the plasma caffeine curve was not predictive of the caffeine cue. Specifically, at four hours post-injection, the differential decline between the plasma caffeine level and the caffeine cue was significant. However, during the absorptive phase of the curve, there was a correspondence between the cue and plasma level. In a previous study, Modrow *et al.* [9] reported that caffeine doses between 5.6 and 32.0 mg/kg resulted in a dose-related generalization to the 32 mg/kg caffeine cue. These individual doses would be expected to produce peak plasma caffeine levels comparable to those seen in the present study 1, 5, and 10 minutes following the injection of 32 mg/kg caffeine. Thus, the caffeine cue appears to be predictive of the plasma level only during the absorptive phase of the plasma curve.

These results would argue that the relationship between caffeine discrimination and the absolute plasma caffeine level is not a simple function. This lack of a simple relationship is not without precedent. The firing pattern of individual reticular formation neurons after caffeine injection exhibited much the same drug level response function as that seen in the present study [2]. After oral caffeine administration, neuronal firing rapidly increased and then gradually decreased. The time to peak firing was prior to the time to peak plasma level and neuronal firing returned to baseline levels prior to the return of plasma levels to baseline.

Although blood is one of the most convenient tissues to sample for caffeine levels, several studies suggest that plasma concentration may not be predictive of brain levels. Thithapandha *et al.* [15] reported plasma levels to decline more rapidly than whole brain levels and Christensen and Whitsett [3] found that the rate of clearance of caffeine from the brain was dependent upon the brain region examined. Another important kinetic property is that the sensitivity of a brain region to caffeine is not simply a function of the tissue level [4]. Caffeine administered at 0.1 to 0.5 mg/kg IV will markedly suppress medial thalamic neuronal activity while much higher doses activate brain stem reticular formation (1 to 5 mg/kg) [4,5] and cortical neurons (10 to 100 mg/kg) [11]. As previously mentioned, reticular neuronal activity will return towards control values while plasma levels are still increasing [2]. Thus, once the anatomical substrate of the caffeine cue is known, a precise parallel between specific brain caffeine levels and the caffeine cue may become evident. However, given the complexity of drug effects on synaptic mechanisms and the rapid adaptation of such mechanisms to drug effects, the search for an exact correspondence between brain levels and the caffeine cue may prove difficult.

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