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Anxiolytic Effect of Caffeine and Caffeine-Clonazepam Interaction: Evaluation by NaCl Solution Intake

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TANG, M., H. KURIBARA AND J. L. FALK. *Anxiolytic effect of caffeine and caffeine-clonazepam interaction: Evaluation by NaCl solution intake.* PHARMACOL BIOCHEM BEHAV **32**(3) 773–776, 1989.—The administration of drugs with anxiolytic action to rehydrating rats augments the intake of 1.5% NaCl solution. In order to clarify the status of caffeine as an anxiolytic agent and its possible interaction with a benzodiazepine having high potency and efficacy in this regard, caffeine (0.78–100 mg/kg) alone and caffeine (0.78–50 mg/kg) plus clonazepam (0.05 or 0.50 mg/kg) injections (IP) were administered to rehydrating rats prior to 1-hr sessions during which they drank 1.5% NaCl solution. When given alone, caffeine, within a particular dose range, and clonazepam at both doses, augmented NaCl solution intake, but when administered in combination, caffeine antagonized the effects of clonazepam.

Benzodiazepine-methylxanthine interaction Caffeine Clonazepam NaCl intake Anxiolytic method

BENZODIAZEPINES and barbiturates that have anxiolytic action in humans increase the intake of NaCl solutions in rehydrating rats (11–13, 29–31), while agents lacking anxiolytic action fail to increase NaCl solution ingestion (21,31). Although several benzodiazepines are currently established as standard anxiolytic agents with long histories of therapeutic use, the status of caffeine's anxiolytic action is controversial. There are reports of caffeine functioning as an effective punishment-attenuating or anticonflict agent (2, 5, 22, 23, 32), while other studies fail to find such action (7, 20, 26). Hence, the first aim of this report was to determine the status of caffeine as an anxiolytic agent by using our standard screening method. The effect of a range of caffeine doses on the ingestion of a 1.5% NaCl solution in rats adapted to a daily, 1-hour rehydration session was explored.

It has been suggested that some of the pharmacological actions of the benzodiazepines may be mediated by adenosine. Specifically, some benzodiazepines may prevent the uptake of adenosine into neural and glial cells, thereby potentiating its depressive action (25). Some adenosine agonists are sedatives and muscle relaxants, while methylxanthines, such as caffeine, act as adenosine-site antagonists (3). If caffeine functions as a benzodiazepine antagonist, then it might be expected to block the anxiolytic action of a benzodiazepine. On the other hand, if caffeine, as some investigators have reported, has anxiolytic efficacy, then caffeine-benzodiazepine combinations might be additive in their anxiolytic action. Consequently, the second aim of this study was to evaluate the interaction of caffeine and clonazepam, the latter agent possessing both a high affinity for "central" benzodiazepine sites and a remarkable potency and efficacy in the NaCl solution ingestion procedure for evaluating anxiolytic action (31).

METHOD

Animals

Eight, adult, albino Holtzman rats (Madison, WI) with an initial mean body weight of 362.4 g (range: 338–388 g) were housed individually in standard Acme stainless-steel cages in a temperature-regulated room. A 12-hr on-off illumination condition was adopted for the duration of the experiment (lights on 0700–1400 hr).

Drugs

Caffeine (Sigma Chemical) was dissolved in an aqueous solution of sodium benzoate at a ratio of 1:0.75 (drug:sodium benzoate). Clonazepam (generously supplied by Dr. Peter F. Sorter, Hoffmann-La Roche, Nutley, NJ) was suspended in an Agent K (BioServ, Frenchtown, NJ) medium (Agent K:water = 1 mg/ml). All injections were administered intraperitoneally and the concentration of the drug was adjusted such that injection volumes were held at 1 ml/kg body weight. All drug solutions were prepared immediately before use.

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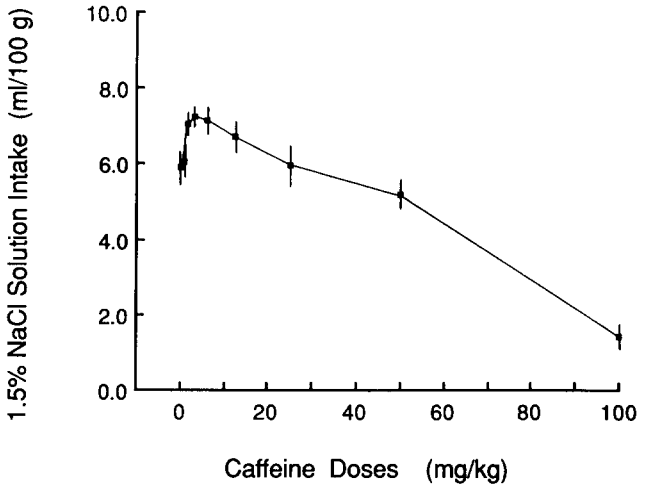

FIG. 1. Mean intake (SE) of 1.5% NaCl solution (ml/100 g) during 1-hr rehydration drinking sessions as a function of caffeine dose (15 min presession, IP).

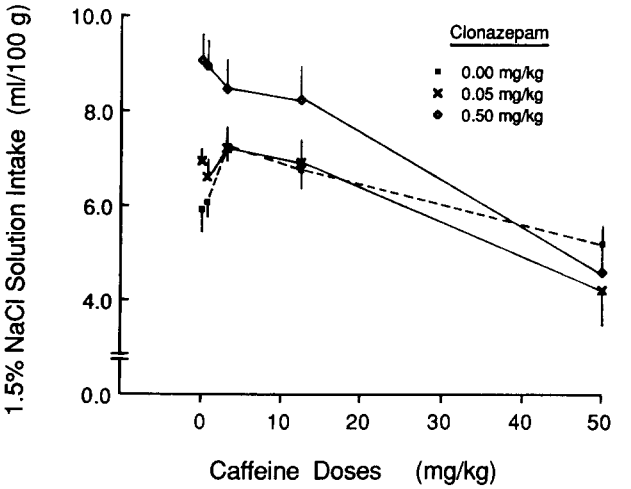

FIG. 2. Mean intake (SE) of 1.5% NaCl solution (ml/100 g) during 1-hr rehydration drinking sessions as a function of caffeine doses (0–50 mg/kg), caffeine doses + either 0.05 or 0.50 mg/kg dose of clonazepam; all doses 15 min presession, IP. The caffeine-alone (0 mg/kg clonazepam) function is plotted from values in Fig. 1 for comparison.

Procedure

All rats were adapted to a 23-hr water-deprivation schedule; access to water was limited to 1 hr for each 24-hr period from a stainless-steel drinking spout (Ancare, TD-300) attached to a 100-ml Nalgene, calibrated cylinder. Food (Purina Laboratory Chow, pelleted) was freely available except during the 1-hr drinking session. At 1000 hr each day, food was removed from the cages, animals were weighed and a filled drinking tube was mounted on the front of each cage. At the end of the 1-hr drinking period, fluid intakes were recorded, the drinking tubes were removed and food was replaced in the cages. After daily session fluid intakes stabilized (14 days), the effects of caffeine given alone, and in combination with clonazepam, on session intakes were determined. Injections were given IP 15 min before the session every 4–6 days. Distilled water was normally the fluid available during the 1-hr drinking session, except on injection days, when a 1.5% (w/w) NaCl solution replaced water as the session fluid. Doses of caffeine were given in two separate injection series (Series I: 0, 12.5, 25, 50 and 100 mg/kg; Series II: 0, 0.78, 1.56, 3.13 and 6.25 mg/kg). The order of the dose given to an animal was randomized within each series. In the caffeine-clonazepam interaction series, the order of the caffeine (0, 0.78, 3.13, 12.5 and 50 mg/kg) and clonazepam (0.05 and 0.50 mg/kg) combination-dose treatments was also randomized.

RESULTS

Figure 1 shows the mean 1-hr intakes (ml/100 g body weight) of 1.5% NaCl solution by rehydrating rats following the administration of various doses (0.78–100 mg/kg) of caffeine 15 min before the drinking session. An overall analysis of variance of the intake data indicates a significant dose-related change in fluid intake, $F(8,56)=31.56$, $p<0.001$. Further analysis reveals that when compared with vehicle treatment, caffeine significantly increased intake at both the 3.13 and 6.25 mg/kg doses, $F(1,7)=19.86$ and 9.01, $p<0.01$ and 0.02, respectively, while the highest caffeine dose used significantly attenuated intake, $F(1,7)=129.32$, $p<0.001$.

The effects of combining clonazepam with various doses of caffeine on 1.5% NaCl solution intake are presented in Fig. 2. Clonazepam alone significantly increased the intake of NaCl solution, $F(2,14)=7.81$, $p<0.01$, but there was no evidence of any summation or potentiation effect of combining clonazepam with caffeine. In fact, caffeine attenuated the clonazepam-induced increase in NaCl solution intake in a dose-related fashion. An overall analysis of variance reveals a significant clonazepam effect, $F(2,98)=15.12$, $p<0.001$. Further tests on individual mean differences show that the 3 dose-effect curves were significantly different only at the 0 (see clonazepam alone F-test above) and 0.78 mg/kg caffeine doses, $F(2,14)=7.98$, $p<0.01$. At greater doses, caffeine antagonized the intake increase produced by clonazepam.

DISCUSSION

Although caffeine displayed significant anxiolytic action as determined by the 1.5% NaCl solution ingestion evaluation method, its efficacy was lower than that of phenobarbital, midazolam, chlordiazepoxide, diazepam or clonazepam (11, 12, 29–31). There is more mention of the anxiogenic property of caffeine (i.e., caffeinism) in clinical and popular literature than of anxiolytic or calming effects [e.g., (15)]. The antiaggressive or calming effects of caffeine were more evident in humans tolerant to caffeine-containing beverages than in those less tolerant (4) and may be related to the reliable preference shown for caffeinated over decaffeinated coffee shown by individuals who were caffeine tolerant/dependent (16). Although of interest, the possibility that the anxiolytic effects of caffeine may be related to a preexisting tolerance/dependence state does not apply to the animals in the present study.

The anxiolytic action of caffeine indicated by the present results agrees with the report by Beer and his associates (2) of anticonflict action for caffeine and other methylxanthines administered intraperitoneally to rats. Morrison (24) and Kuribara and his associates (22) reported increases in punished responding by some rodents when given large doses of caffeine subcutaneously. Caffeine was also effective in increasing punished responding in the squirrel monkey (5,32). On the other hand, no anticonflict activity was obtained for rats administered caffeine (7, 20, 26) or theophylline (7). The differences among these reports cannot at present be reconciled.

In relative independence of whether the administration of

caffeine alone has either a modest anxiolytic effect, or no such effect, the question of the neuropharmacological nature of the caffeine-benzodiazepine interaction and its behavioral result has been the subject of several studies. Conflict studies have reported both additive, anticonflict action when caffeine and a benzodiazepine were administered together (2, 5, 32) and an absence of effect or antagonism (7,26). With respect to other behavioral measures, impaired psychomotor performance produced by benzodiazepines in humans was antagonized by caffeine (14,27) and by theophylline (17). The decreased water intake produced by a large dose of caffeine (30 mg/kg, IP) in water-deprived rats was antagonized by the administration of either diazepam or midazolam (8). Finally, chlordiazepoxide blocked the caffeine stimulus in rats trained to discriminate caffeine stimulation from vehicle in a drug discrimination procedure, although the chlordiazepoxide stimulus was not antagonized by caffeine (18). Together with the present results, which gave no indication of additivity, the evidence favors an antagonist interaction between the anxiolytic benzodiazepines and the methylxanthines.

The pharmacological basis of the antagonism reflected in behavioral measures is by no means clear. Although methylxanthines can inhibit the binding of [^{3}H]diazepam in brain tissue, they do so in vitro at high concentrations beyond behavioral relevance (1,23), nor can pharmacokinetic interactions account for the antagonism (17). The suggestion that the anxiolytic action of the benzodiazepines may be due to their blockade of adenosine uptake in the brain (25) appears doubtful insofar as the doses required, particularly for clonazepam, for effective action may be beyond physiological relevance (3,33). Further, only scant evidence (28, 34) suggests [along with negative evidence (9,10)] that adenosine agonists might possess anxiolytic action. Hence, although the depressive behavioral and cardiovascular effects of adenosine analogs can be antagonized by caffeine doses that alone have no effect (6), it is doubtful that the antagonism is receptor-site specific (19). Inasmuch as physiological concentrations of adenosine do not affect benzodiazepine receptors, nor do physiological concentrations of benzodiazepines affect adenosine receptors (3), it is unlikely that caffeine-benzodiazepine interactions can be reduced to mediation by adenosine mechanisms.

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