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Caffeine-Induced Hypodipsia in Water-Deprived Rats: Relationships with Benzodiazepine Mechanisms

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COOPER, S. J. *Caffeine-induced hypodipsia in water-deprived rats: Relationships with benzodiazepine mechanisms.* PHARMAC. BIOCHEM. BEHAV. 17(3) 481–487, 1982.—The effects of caffeine (3–100 mg/kg) on water intake and the time course of drinking were investigated in male rats which had been adapted to a daily 22 hr water deprivation schedule. Doses of caffeine were found which significantly depressed water intake, reduced the time to the first interruption in drinking, and depressed the time course of drinking, without manifestly affecting the efficiency of drinking. At the highest dose, however, caffeine had a major suppressant effect on drinking, which was accompanied by signs of motor interference. The hypodipsic effect of caffeine was reversed by benzodiazepine treatment (midazolam or diazepam). However, the convulsant benzodiazepine Ro5-3663 which on electrophysiological evidence can act as a GABA antagonist also reduced drinking, adding to the hypodipsic effect of caffeine. A water load prior to the drinking test produced satiation effects, closely reminiscent of the effects of caffeine at lower doses. The possible mimicry of thirst satiety by caffeine is discussed, together with possible underlying mechanisms of caffeine-benzodiazepine interactions.

Caffeine Diazepam Drinking Midazolam Ro5-3663 Thirst Satiety

CAFFEINE, a methylxanthine, has recently been shown to act antagonistically with respect to certain characteristic pharmacological actions of the benzodiazepines. The seizures induced in mice by high doses of caffeine (300–350 mg/kg) are antagonized by diazepam, flunitrazepam and clonazepam [19]. High doses of caffeine are effective in antagonizing the anticonvulsant effect of diazepam in pentylenetetrazol-induced seizures in mice [26]. At much lower doses, caffeine abolishes the anticonflict effect of diazepam in rats [26]. The main aim of the present series of experiments was to determine whether caffeine acts in an antagonistic sense to benzodiazepines, in relation to an unpunished consummatory response, thirst-induced drinking.

It is probable that benzodiazepine mechanisms serve an important modulatory function in the control of water intake in the water-deprived rat [6,7]. Thus, a variety of benzodiazepines show a consistent action to increase the volume of water consumption in thirsty rats [5, 6, 7, 14, 15, 16, 21, 28, 31]. Furthermore, the hyperdipsic effect of benzodiazepines can be blocked by the action of Ro15-1788 (Cooper, unpublished data). Ro15-1788 is a new specific antagonist of benzodiazepine action at the benzodiazepine receptor [13, 22, 27]. The enhanced water intake which follows benzodiazepine treatment is not dependent upon the action of a punisher [21], novelty [31], or sedation [7]. The increased intake follows as a result of an extension in the time-course, or duration, of drinking as the thirsty rat drinks to repletion [6, 7, 31]. The hyperdipsic effect of the benzodiazepines may reflect a relatively direct involvement with signals of either thirst or its satiety in the rat.

The first experiment reported here examined the possibility that caffeine reduces water consumption in thirsty rats. There is some evidence that caffeine, at a relatively high dose, can reduce schedule-induced polydipsia [32]. However, evidence did not seem available concerning its effects on deprivation-induced drinking. The second experiment tested the interactions between caffeine and two benzodiazepines, diazepam and a new, water-soluble imidazobenzodiazepine, midazolam [9,29]. The proconvulsant benzodiazepine Ro5-3663 [30] differs in its electrophysiological effects from the conventional depressant or anxiolytic benzodiazepines. The third experiment therefore investigated the possibility that this compound produces a converse effect on drinking (i.e., to reduce water intake in the thirsty rat). If so, it might act in concert with caffeine. The final experiment attempted to match the effects of caffeine on drinking behavior by water-loading the rats before the drinking test period. The results of these studies showed that caffeine induced a hypodipsia in the water-deprived animals, and that this effect was blocked by concurrent treatment with diazepam or midazolam. On the other hand, the convulsant benzodiazepine Ro5-3663 acted additively with caffeine

to reduce water intake. The finding that the effects of caffeine could be matched by prior water-loading, suggested that caffeine may mimic thirst-satiety signals.

EXPERIMENT 1

METHOD

Animals

The subjects were 40 male hooded rats bred in the laboratory. They were housed individually in stainless steel cages, with free access to food pellets (Diet 41B, Heygate and Sons, U.K.) at all times. They were maintained under a 12 hr light-12hr dark cycle (lights on a 7 a.m.) and room temperature was kept constant at 21°C. The animals had been adapted to a daily 22 hr water-deprivation schedule for several weeks before the experiments began in order to ensure stable baseline water intake and drinking patterns. Care was taken to familiarize the animals completely with the relevant experimental procedures before running the drug trials. They were handled and weighed regularly, and they had received experience of intraperitoneal injections of isotonic saline. They weighed between 350–450 g at testing.

Procedure

For the drinking test, a calibrated cylinder containing tap-water was fixed to the front of the cage, with the metal spout protruding into the interior. Half the animals were given their daily 2 hr access between 1000 a.m. and noon, and the remaining half were given their access between 0230 and 0430 p.m. The volume of water consumed during the first 20 min access to water (during which time most of the daily water requirement was ingested) was determined to the nearest 0.5 ml by reading the level in the calibrated cylinder. The time-course of drinking was determined for each rat by noting, at 15 sec intervals throughout the 20 min test period, whether or not the animal was drinking. Taken across a group of animals, this time-sampling method yielded a measure of the frequency of animals drinking at regular time intervals. These time-course data could also be used to estimate the actual time devoted to drinking within the test. The estimate of drinking duration was obtained for each rat by dividing the number of intervals on which drinking was observed by 4, to give a total time (in minutes) devoted to drinking. The reliability of the estimate was determined for a group of 16 rats by measuring the actual duration of drinking over the 20 min test period using a cumulative timer, and comparing the results with the estimated duration of the drinking obtained by the time sampling method. A correlation coefficient $r=0.94$ was obtained, with a mean error of $<0.1\%$. From the two measures of the volume of water intake and the duration of drinking, a value for the local rate of drinking (ml/min) could be calculated. This measure was calculated in order to yield a general index of the efficiency of drinking, to detect any disruption, depression or enhancement of the rate of ingestion. A further measure used was the number of 15 sec intervals for which drinking was continuously in progress from the start of the drinking test, i.e., the time to the first pause or interruption of drinking. This measure proved sensitive to caffeine's effect. A note was always made of the behaviors which were exhibited (e.g., grooming, activity) whenever drinking was interrupted or had stopped.

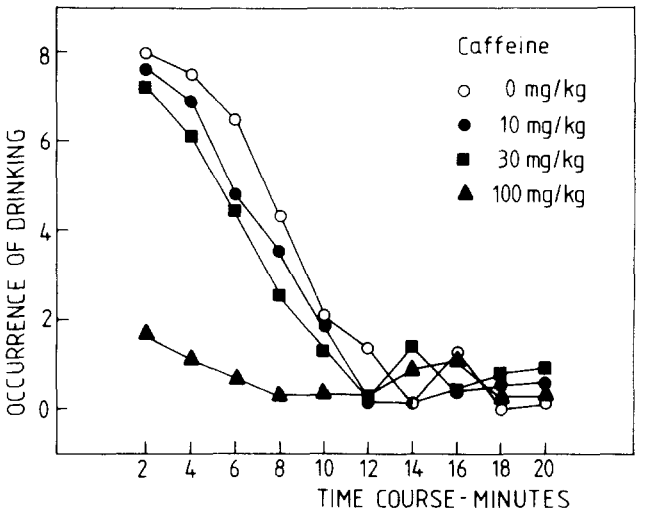

FIG. 1. Effects of caffeine on the time-course of drinking in the water-deprived rat over a 20 min observation period. The number of occasions on which each rat was observed to drink at 15 sec intervals was scored. The maximum score for the occurrence of drinking was 8 per 2 min interval. Curves indicate mean results ($N=8$ per group). The dosage conditions are identified by the key. The results for 3 mg/kg caffeine were indistinguishable from the control data, and are therefore omitted for clarity.

For the first experiment, the rats were randomly assigned to 5 equal groups, and within each group they were balanced according to morning or afternoon access to water. The groups were allocated to 5 injection conditions: 3, 10, 30 and 100 mg/kg caffeine (obtained from Sigma, London), and an isotonic saline vehicle. All injections were administered IP, 30 min before the start of the drinking test.

Drug effects were assessed by comparing drug-treated groups with the control group using a *t*-test for independent means.

RESULTS AND DISCUSSION

Following a vehicle injection, the water-deprived rats began to drink as soon as the water was returned to the home cage. They typically drank continuously for 4–5 min (16–20 observation periods at 15 sec intervals), before showing a rapid decline in the occurrence of drinking (Fig. 1). During the second half of the 20 min test period, the frequency of drinking remained at a relatively constant low level. Figure 1 depicts the time-courses of drinking for the control and caffeine conditions in terms of the frequency of rats observed to be drinking throughout the test period.

Caffeine (3–30 mg/kg) had no effect on the latency to begin drinking. All animals began to drink immediately after the drinking tube was restored to the home cage. Over this dose range, there was a dose-related effect on the time-course of drinking (Fig. 1). Over the first half of the test period, when drinking dropped from its initial high level most rapidly, caffeine depressed the time-course relatively evenly throughout. There was little to discriminate amongst the injection conditions during the second half of the test period, when the frequency of drinking occurrence remained at a low level.

Caffeine (100 mg/kg) had a major suppressant effect on

TABLE 1

EFFECTS OF CAFFEINE ON WATER INTAKE AND DRINKING PARAMETERS IN WATER-DEPRIVED RATS TESTED ON A 20 MIN DRINKING TEST

Caffeine (mg/kg)	N	Water intake (ml)	Rate of intake* (ml/min)	Number of intervals to first drinking pause†
0	8	20.4 ± 1.0	2.7 ± 0.1	18.4 ± 1.9
3	8	19.9 ± 1.3	2.7 ± 0.1	13.0 ± 3.4
10	8	17.7 ± 1.1	2.8 ± 0.2	8.6 ± 1.9§
30	8	14.5 ± 0.7§	2.3 ± 0.2	6.3 ± 1.7§
100‡	8	2.8 ± 1.0§	—	—

Results are shown as mean ± S.E.M.

*Calculated in terms of the water intake divided by the estimated duration of drinking (min).

†Number of 15 sec intervals that rats drank continuously from the start of the test to the first interruption in drinking.

‡Since rats were seriously impeded by the highest caffeine dose, the two drinking parameters were not calculated.

Stastical comparisons: Level of significance §$p<0.005$.

TABLE 2

EFFECTS OF EITHER MIDAZOLAM OR DIAZEPAM ON CAFFEINE-INDUCED HYPODIPSIA IN WATER-DEPRIVED RATS

Injection condition	N	Water intake (ml)	Number of intervals to first drinking pause
Vehicle	8	20.6 ± 0.9	22.3 ± 3.0
Caffeine 30 mg/kg	8	14.3 ± 1.1†	7.5 ± 2.0†
Midazolam 1.25 mg/kg	8	23.4 ± 1.0*	30.6 ± 4.0
Caffeine 10 mg/kg + Midazolam 1.25 mg/kg	8	22.1 ± 1.0	24.0 ± 2.3
Caffeine 30 mg/kg + Midazolam 1.25 mg/kg	8	19.9 ± 1.4	14.0 ± 3.1
Vehicle	8	20.1 ± 1.6	18.0 ± 3.3
Diazepam 1.25 mg/kg	8	21.5 ± 0.7	24.5 ± 2.0
Caffeine 10 mg/kg + Diazepam 1.25 mg/kg	8	19.7 ± 1.0	16.0 ± 3.7
Caffeine 30 mg/kg + Diazepam 1.25 mg/kg	8	19.6 ± 1.1	18.1 ± 3.4

Results are shown as mean ± S.E.M.

*$p<0.05$; †$p<0.005$.

drinking. Only 3 of the 8 animals treated began drinking immediately. Drinking occurred at an unusually low level throughout the test (Fig. 1). Five of the 8 animals drank 1.5 ml or less in the 20 min test period. Observation indicated that the behavioral effects of caffeine at this high dose were qualitatively different from those at the smaller doses. All rats treated with caffeine at 100 mg/kg showed marked sedation, lying on the floor bars with little movement. Subsequent tests revealed them to be underresponsive to a variety of stimuli (touch, pain, sound and visual), although they could be aroused to a small degree by handling. Attempts to drink from the drinking spout during the drinking test were often abortive (e.g., raising mouth to tube but failing to lick). None of these characteristics were noted in animals injected with 30 mg/kg caffeine or less.

Table 1 indicates the effect of caffeine on water intake (ml) in the test. There was a dose-related decrease in water consumption, with a highly significant 29.4% reduction occurring at 30 mg/kg. As noted above, caffeine at the high dose of 100 mg/kg almost completely suppressed drinking. A measure of the efficiency of drinking can be gained from an estimate of the local rate of drinking (obtained from the water intake and the total number of occasions on which drinking was observed to take place). There was no evidence that caffeine (3–30 mg/kg) exerted any significant effect on the efficiency of drinking, measured in terms of the rate of water intake (Table 1). One parameter that was sensitive to caffeine's effects at low doses was the number of intervals to the first pause in drinking. Control rats drank continuously for a mean 18.4 intervals of 15 sec from the start of the drinking test to the first interruption in drinking (defined as at least one observation interval without drinking). Caffeine (10 and 30 mg/kg) produced highly significant reductions in this initial period of continuous drinking (Table 1). Both control and caffeine-treated rats broke off from drinking in order to groom (observed across all animals), but the caffeine-treated animals interrupted their drinking sooner. The significance of this difference is taken up later in relation to Experiment 4.

Summarizing, therefore, caffeine reduced water-intake, and this effect occurred without change in the latency to begin drinking or in the efficiency of consumption whilst drinking was in progress. The reduction in water intake was associated with a dose-related decline in the frequency of occurrence of drinking (Fig. 1). At a relatively high dose (100 mg/kg), caffeine markedly disrupted behavior, and produced a form of sedation which appeared to be incompatible with drinking. This suggests some caution in the interpretation of caffeine's effects on consummatory behavior which occur at higher doses [32]. The aim of the next experiment was to test for caffeine-benzodiazepine antagonism, by attempting to block caffeine's effects on drinking behavior using either diazepam or midazolam.

EXPERIMENT 2

METHOD

The subjects were the 40 animals used in Experiment 1. They were housed and maintained on a daily 22 hr water-deprivation schedule as before. The 20 min drinking test was carried out as described in the previous experiment. The animals were randomly assorted to 5 equal groups, and were assigned to the following injection conditions: 30 mg/kg caffeine; 1.25 mg/kg midazolam bimaleate; 10 mg/kg caffeine and 1.25 mg/kg midazolam; 30 mg/kg caffeine and 1.25 mg/kg midazolam; isotonic saline vehicle. Solutions were made up with isotonic saline, and all injections were administered IP, 30 min before the start of the drinking test.

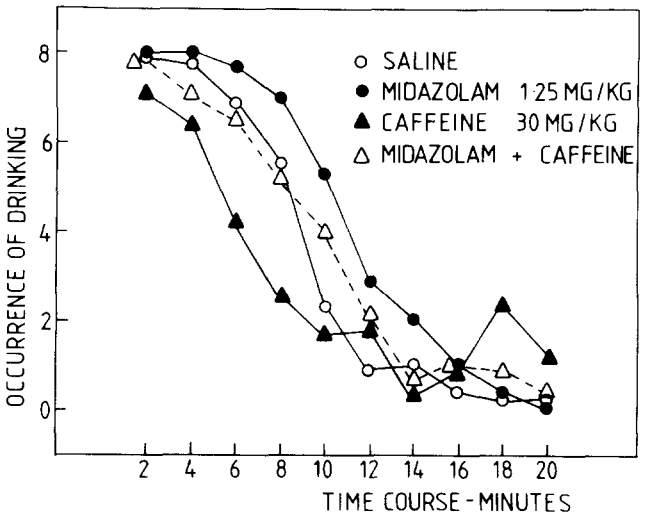

FIG. 2. Midazolam-caffeine antagonism depicted in terms of the time-course of drinking in the water-deprived rat over a 20 min test period. Curves indicate mean scores for the occurrence of drinking at 15 sec intervals over consecutive 2 min intervals (N=8 per group). The injection conditions are identified by the key.

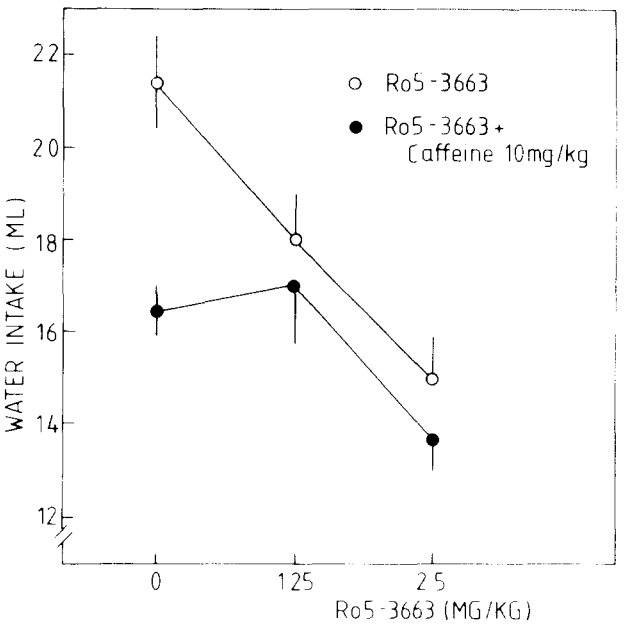

FIG. 3. Effects of caffeine (10 mg/kg) and the convulsant benzodiazepine Ro5-3663 on water intake (ml) in the water-deprived rat over a 20 min test period. Results are shown as means±SEM (some standard error bars are omitted for clarity). Both caffeine and Ro5-3663 significantly attenuated water intake; their joint effects were additive.

One week after completion of this testing, 32 of the animals were randomly assigned to 4 equal groups, and retested on the drinking test. These 4 groups were allocated to the following injection conditions: 1.25 mg/kg diazepam; 10 mg/kg caffeine and 1.25 mg/kg diazepam; 30 mg/kg caffeine and 1.25 mg/kg diazepam; a vehicle condition of 48% propylene glycol; 52% distilled water, which was also used to dissolve the diazepam. The injections were given IP, 30 min before the start of the drinking test.

The data were analysed by comparing the results for drug injections conditions with the corresponding control group using a *t*-test for independent groups.

RESULTS AND DISCUSSION

Confirming the results of Experiment 1, caffeine (30 mg/kg) significantly reduced water intake in the 20 min drinking test, and also reduced the number of intervals before rats first interrupted their drinking in order to groom (Table 2). Caffeine (10 and 30 mg/kg) in combination with either midazolam (1.25 mg/kg) or diazepam (1.25 mg/kg) had no significant effects on drinking (Table 2). Hence, low dose treatment with benzodiazepines antagonized the hypodipsic effect of caffeine. Figure 2 shows the extension in the time-course of drinking that occurred after midazolam treatment, the reduction in the frequency of drinking produced by caffeine (30 mg/kg), and the mutual antagonism that occurred when the two drugs were administered concurrently.

Thus, the antagonism of caffeine's effects on thirst-induced drinking by the benzodiazepines is fully in accord with other recent evidence for caffeine-benzodiazepine antagonism [19,26]. The present data extend previous findings to include unpunished drinking responses. If benzodiazepine mechanisms are involved in the modulation of thirst-induced drinking responses [6,7], then caffeine does appear to act in opposition to the benzodiazepine effects. This notion would be supported if caffeine acts in concert with the atypical proconvulsant benzodiazepine Ro5-3663 [30], which on electrophysiological evidence behaves antagonistically with respect to the actions of the conventional depressant compounds [30].

The aims of the third experiment were two-fold. Firstly, the prediction that Ro5-3663 should, in contrast to the depressant benzodiazepines, retard drinking in thirsty rats was examined. Secondly, the combined effects of caffeine and Ro5-3663 were investigated.

EXPERIMENT 3

METHOD

The subjects were 48 male hooded rats, 40 of which had been used previously. The additional 8 animals had also been well adapted to the 22 hr daily water-deprivation schedule, and had been thoroughly accustomed to the handling and injection procedures. The animals were housed and run in the drinking test, as described for the first experiment.

The study was designed as a 2×3 factorial experiment, consisting of 6 groups of 8 animals each. Each animal received two injections. Half the rats received an IP injection of 10 mg/kg caffeine, and the remaining half received a control saline injection, 30 min before the start of the drinking test. Within each of these two groups, the animals were subdivided into 3 groups. The injection conditions for these groups were:1.25 mg/kg Ro5-3663 (1,3-dihydro-5-methyl-2H-1,4-benzodiazepine-2-one), 2.5 mg/kg Ro5-3663, and a vehicle group (48% propylene glycol: 52% distilled water). The chosen doses were within the subconvulsant range. The second injection immediately followed the first.

The water intake data were analysed using analysis of variance (ANOVA) procedures [33], which permitted an

assessment of the separate effects of the two drugs, caffeine and Ro5-3663 on deprivation-induced drinking, and the nature of any interaction which might occur when the two drugs were administered in combination.

RESULTS AND DISCUSSION

Figure 3 depicts the effects of caffeine and Ro5-3663 alone and in combination on thirst-induced water intake. Both caffeine, $F(1,42)=8.97$, $p<0.005$, and Ro5-3663, $F(2,42)=10.38$, $p<0.001$, significantly attenuated water consumption. Thus the convulsant benzodiazepine, like caffeine but in contrast to conventional, depressant benzodiazepines, reduced drinking in the thirsty animals. The ANOVA interaction term was not significant, $F(2,42)=2.18$. Hence, caffeine and Ro5-3663 exerted hypodipsic effects which were essentially additive. Thus, the presence of the convulsant benzodiazepine did not modify the action of caffeine, and vice-versa. No drug treatment affected the latency to begin drinking. All animals started to drink as soon as water was returned to the home cage.

Electrophysiological evidence shows that Ro5-3663 selectively blocks the effect of γ-aminobutyric acid (GABA) at spinal and peripheral neuronal sites [30]. This action is in marked contrast to that of the depressant or anxiolytic benzodiazepines, which typically facilitate GABAergic transmission at such sites, and at supraspinal locations [8, 11, 12, 29]. The present study provides behavioral evidence that Ro5-3663, at subconvulsant doses, exerts an opposite effect on thirst-induced drinking to that typically obtained with the more familiar benzodiazepines. A possible explanation for Ro5-3663's hypodipsic effects is a blockade of GABAergic transmission which helps to mediate drinking responses in thirsty rats. This interpretation is consistent with the finding that the GABA antagonist, picrotoxin, also depresses deprivation-induced drinking at subconvulsant doses [5].

These results show that caffeine can act in unison with Ro5-3663 to bring about a reduction in drinking, whilst caffeine-induced hypodipsia can be antagonized by depressant benzodiazepines, such as midazolam or diazepam (Experiment 2).

EXPERIMENT 4

One mechanism by which caffeine might reduce thirst-induced drinking would be to mimic thirst satiety, and thereby at least partially suppress drinking. The effects of caffeine should therefore be matched by allowing a certain degree of satiation to take place prior to the drinking experiment. The aim of this final study was to investigate the volume of water intake and the time course of drinking in the drinking test, after the animals had been allowed a prior water load of either 6 or 11 ml.

METHOD

Forty-eight rats used in previous studies were subjects. They were housed and maintained as before. They were randomly allocated to 3 equal groups. Two of the groups were allowed to drink a set amount of water, 30 min before the drinking test itself. The first of these was allowed 6 ml, in an attempt to match roughly the decrease in water intake produced by 30 mg/kg caffeine (Experiment 1). The second was allowed access to 11 ml. In both groups, the water load was consumed continuously and with negligible latency. The third group had no access to water prior to the drinking test.

TABLE 3

EFFECTS OF ALLOWING ACCESS TO EITHER 6 OR 11 ml WATER PRIOR TO A 20 MIN DRINKING TEST IN PREVIOUSLY WATER-DEPRIVED RATS

Water pre-load (ml)	N	Water intake (ml)	Rate of intake (ml/min)	Number of intervals to first drinking pause
0	16	17.2 ± 0.8	2.4 ± 0.1	19.4 ± 2.2
6	16	15.9 ± 0.9	2.4 ± 0.1	20.1 ± 1.7
11	16	9.4 ± 0.7*	2.2 ± 0.1	8.4 ± 0.9*

Results are shown as mean ± S.E.M.
*$p<0.005$.

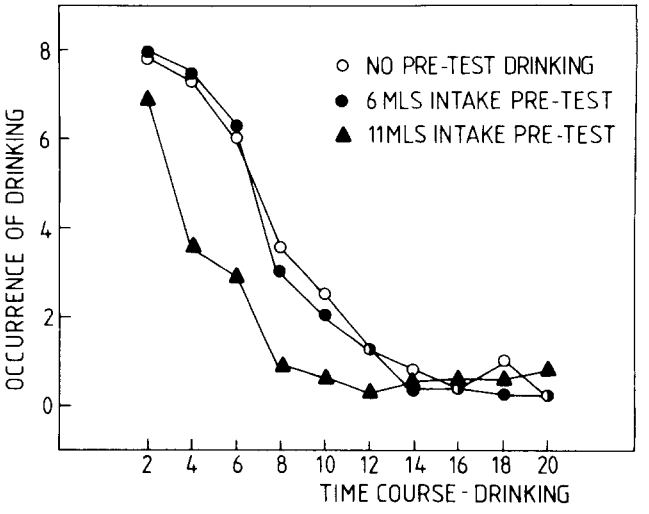

FIG. 4. Satiating effects of water loads ingested 30 min before the 20 min drinking test. Curves indicate mean scores for the occurrence of drinking at 15 sec intervals over a 20 min test period (N=16 rats per group). The pre-test conditions are identified by the key. A marked depression in the occurrence of drinking (reflecting thirst satiation) took place after a load of 11 ml water.

In the 20 min test which followed, water intake, the number of intervals to the first pause in drinking, and the time-course of drinking were recorded, as described for the first experiment.

RESULTS AND DISCUSSION

Prior access to 6 ml water produced a slight, although statistically insignificant reduction in water intake in the drinking test, and no alteration in the number of observation intervals to the first interruption in drinking (Table 3). There was correspondingly little change in the time-course of drinking (Fig. 4). However, prior access to 11 ml water did produce a prominent thirst satiation effect. It produced a highly significant drop in the water intake during the 20 min test period, and a similar reduction in the number of intervals to the first pause in drinking (Table 3). The frequency of occurrence of drinking was markedly suppressed, particu-

larly during the first half of the test. The 6 ml or 11 ml water loads did not however affect the estimated rate of water intake in the drinking test (Table 3). It is note-worthy that the satiating effect (measured as the reduction in water intake in the drinking test) of the water loads was typically less than the load itself (Table 3).

These results can be compared with those obtained with caffeine (Experiment 1). It is clear that there are close similarities between the effects of caffeine and a prior water load exceeding 6 ml. Thus caffeine at 30 mg/kg also reduced water intake, reduced the number of intervals to the first pause in drinking, and depressed the time-course of drinking over the first half of the drinking test. The significant effects of the 11 ml water load can be attributed to thirst-satiety; the rats having partially satisfied their water requirement, showed a smaller intake in the drinking test period and a reduced frequency of drinking which was reflected in the depressed time-course curve shown in Fig. 4. By analogy, caffeine treatment appears to act like thirst-satiety. As far as drinking is concerned, caffeine may therefore act as a satiety-mimetic agent.

GENERAL DISCUSSION

Caffeine at the relatively low doses of 10 and 30 mg/kg, significantly reduced deprivation-induced drinking in rats (Experiments 1 and 3). The caffeine-induced hypodipsia followed from a suppression of the time-course of drinking, but did not depend on either an increase in the latency to begin drinking or a decrease in the estimated rate of water intake whilst drinking was in progress (Experiment 1). Caffeine-treated animals broke off from drinking sooner, after the start of the drinking test, in order to engage in grooming activity. In all respects, these effects were closely similar to the effect observed following water-loading of 11 ml before the drinking test (Experiment 4). The close correspondence strongly suggests that caffeine can mimic partial thirst-satiation in the water-deprived rat. There is a limit to the dose of caffeine which can be usefully employed in these experiments. Caffeine at 100 mg/kg produced a behavioral depression which effectively interfered with drinking (Experiment 1).

The caffeine-induced hypodipsia was antagonized by low dose treatment with either midazolam or diazepam (Experiment 2). These results corroborate other evidence for caffeine-benzodiazepine antagonism [19,26]. The central mechanisms of action of the methylxanthines have been recently reviewed [3,10]. Purinergic transmission has been implicated, since caffeine has been shown to antagonize the effects of adenosine on synaptic transmission in the cerebral cortex [25]. The fact that diazepam shows the opposite effect, that is to potentiate purinergic effects on cerebral cortex neurons [24], provides one explanation for the observed caffeine-benzodiazepine antagonism at a behavioral level. An alternative explanation invokes actions at specific benzodiazepine binding sites in the central nervous system. The methylxanthines, caffeine, theophylline and theobromine, all competitively inhibit H^3-diazepam binding [1, 17, 18], and inhibit GABA-stimulated diazepam binding with higher potency [20]. Caffeine-benzodiazepine antagonism might therefore arise as a consequence of competition at the level of benzodiazepine binding sites.

Some earlier behavioral work indicated a possible anxiolytic action of the methylxanthines, identified using a type of "conflict" procedure [2]. This may not be a reliable finding [4], and recent work has demonstrated that the anti-conflict action of benzodizepines can be antagonised at low doses of caffeine [26]. The present data show that when unpunished responses are utilised, caffeine-benzodiazepine antagonism can also be demonstrated.

Binding studies have shown that the convulsant benzodiazepine Ro5-3663 binds competitively to α-dihydroxypicrotoxinin (a picrotoxin analogue) binding sites with a higher affinity than for the high affinity benzodiazepine sites [23]. Binding to such sites may underlie the similarity between Ro5-3663 and picrotoxin [5] in reducing thirst-induced drinking at subconvulsant doses. Recently however, Ro5-3663 has been shown to inhibit GABA-stimulated ^{3}H-diazepam binding at concentrations which have no effect on basal binding [20]. Under conditions of GABA stimulation therefore, Ro5-3663 may act as an effective benzodiazepine antagonist at benzodiazepine binding sites. Additional pharmacological work should help to determine the mechanisms by which Ro5-3663 exerts its functional effects.

In summary, there is behavioral evidence with regard to the drinking response elicited in the thirsty rat that caffeine and benzodiazepines exert opposite effects. The caffeine-induced hypodipsia, which may depend upon thirst-satiety signals, was blocked by concurrent treatment with benzodiazepines. Caffeine and the convulsant benzodiazepine, Ro5-3663, which may share fundamental actions at binding sites, behaved additively in bringing about a satiation of drinking responses. Hence, continued investigation of methylxanthines, benzodiazepines and related compounds may prove of great significance in understanding the basis of the drinking response in the water-deprived rat.

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