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Evidence That Agents Increasing Water Consumption Do Not Necessarily Generate "False Positives" in Conflict Procedures Using Water as a Reinforcer

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CARLI, M. AND R. SAMANIN. *Evidence that agents increasing water consumption do not necessarily generate "false positives" in conflict procedures using water as a reinforcer.* PHARMAC. BIOCHEM. BEHAV. 17(1) 1–3, 1982.—Isopreterenol 7.5 and 15 μg/kg prolonged the time spent in drinking of water-deprived rats whereas 30 μg had no significant effect. No dose of isoproterenol affected punished responses in a conflict procedure which uses water as reinforcer. Punished responses were increased by 2.5, 5 and 10 mg/kg of diazepam but only the first two doses of diazepam increased drinking in normal deprived rats. The data show that the effects on punished responses and drinking can be dissociated and suggest that procedures using conditioned punishing of consummatory responses may be more appropriate than those using naive animals for revealing "true" antipunishment activity.

Isoproterenol Drinking Anticonflict effects

ISOPROTERENOL, a beta adrenergic agent which increases drinking is satiated rats [9] has been shown recently to increase punished responses in a test in which the required response was licking from a drinking tube [8]. Since isoproterenol does not seem to have anti-anxiety effects [7], these findings were taken as evidence that agents affecting primary drive states may generate false positives in conflict procedures aimed at testing antianxiety activity.

A consummatory conflict procedure, which uses water as reinforcer and is sensitive to various benzodiazepines has been recently developed in our laboratory [2,3]. This test, unlike that used by Patel and Malick, uses trained rats and permits assessment of punished and unpunished responding in the same animal in a single test session.

In an attempt to distinguish between effects on punished responses and those on primary drive states, we compared the effects of isoproterenol and diazepam on punished and unpunished responding in this test, and on drinking [8] of water-deprived rats not trained in the conflict procedure. Caffeine was also included in the experiments since it has been reported to enhance punished consummatory responses in some tests [1] but not in others [5].

METHOD

Female CD-COBS rats (Charles River, Italy) weighing 200–250 g at the beginning of the experiments were used. The animals were maintained at constant room temperature (22±1°C) and relative humidity (60%). During the experiments rats were deprived of water except for 60 minutes of free access to water in their home cage one hour after each daily experimental session. Food was available ad lib.

Apparatus

The experimental chamber was rectangular box (30×30×40 cm) with plastic sides and top and a grid floor. Through a hole in the middle of one wall, 4 cm in diameter and 8 cm above the grid floor, the rat had access to a stainless steel drinking tube, 7 mm in diameter, recessed 2 cm from the plane of the cage wall and connected to a plastic bottle containing approximately 100 ml of water. The support for the drinking tube contained a metal clip and leads connected to a shock device, whose current was adjustable from 90 to 440 μA AC at 220 V. The grid floor comprised the other pole to complete the shock circuit through the animal's paws. Thus only animals licking the tube received a shock under conflict condition. Through a speaker mounted in the ceiling of the cage a tone signal was presented. The sensing device for the rat's contacts with the drinking tube was a photocell set horizontally in the tube support, its beam just clearing the end of the tube. The photocell beam was narrow (less than 2 mm) making it unlikely that the animal could break it without contacting the drinking tube. Direct observation indicated that in fact the beam was broken only when rats contacted the tube.

A digital programmer was used to control experimental contingencies and record responses. Three separate timers controlled cycles of tone, tone-plus-shock, and silent period (no shock). Digital clocks recorded the total time of inter-

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TABLE 1

EFFECTS OF DRUGS ON DRINKING OF WATER-DEPRIVED RATS IN "CONFLICT" AND "NON CONFLICT" CONDITIONS

Drug	Drinking time (% of control)		
	"non conflict"	"conflict"	
		punished response	unpunished response
Isoproterenol			
7.5 μg/kg	135 ± 7†	82 ± 20	116 ± 10*
15.0 μg/kg	125 ± 7*	105 ± 19	117 ± 7†
30.0 μg/kg	110 ± 7	105 ± 25	109 ± 10
Diazepam			
2.5 mg/kg	133 ± 6*	187 ± 36*	74 ± 13
5.0 mg/kg	139 ± 5*	368 ± 43*	79 ± 15
10.0 mg/kg	118 ± 7	251 ± 54*	70 ± 14
Caffeine			
10.0 mg/kg	—	95 ± 27	89 ± 10
50.0 mg/kg	—	71 ± 18	68 ± 7*

Ten animals were used for each dose of the various compounds, except for diazepam in "conflict" experiments where 7 animals were used for each dose.

Effects on treatment day are expressed as percentages (±S.E.M.) of the mean baseline value for the controls tested the day before each treatment.

Diazepam (intraperitoneally), isoproterenol (subcutaneouly) and caffeine (orally) were administered respectively 15, 30 and 60 minutes before testing.

Statistical analysis was made on actual data: $*p<0.05$, compared with controls, $\dagger p<0.01$, compared with controls.

ruption of the photocell beam for each period. An electromechanical counter recorded the number of shock episodes (a make-break contact on the drinking tube during the period of tone-plus-shock).

Procedure

Cycles of 3 sec (tone), 10 sec (tone-plus-shock) and 20 sec (silent period) were used. Daily sessions lasted 10 minutes, yielding about 20 cycles of the sequence tone alone, tone-plus-shock and silent period. During training, water-deprived rats were placed in the cage without delivery of electric shock, to locate the drinking tube and become accustomed to drinking from it. After drinking behaviour had stabilized (about 10 days of training), the schedule was presented with the shock level set at 180 μA. On the basis of the level of suppressed drinking for each rat, the shock level was adjusted each day to achieve a similar range of suppressed responding in all subjects (about 50% of maximal contact time exhibited by each rat during training without shock). This intermediate degree of suppression was chosen as preliminary experiments had shown it is the most appropriate to reveal significant increases or decreases in suppressed responding. The animals were tested for drug effects when they had reached fairly stable responses during 10-minute sessions (about 10 days). In some experiments water-deprived rats were trained in the same apparatus using the same schedule but with no electric shocks at any time (non conflict). Drug effects on 10-minute drinking were assessed in these animals.

Drugs

Diazepam was administered intraperitoneally at 2.5, 5 and 10 mg/kg, 15 minutes before testing. Isoproterenol hydrochloride (7.5, 15 and 30 mg/kg) was administered subcutaneously and caffeine (10 and 50 mg/kg) orally, respectively, 30 and 60 minutes before testing. Diazepam was suspended in carboxymethylcellulose 0.5% (CMC). Isoproterenol and caffeine were dissolved in distilled water. Controls received distilled water (isoproterenol or caffeine treated rats) or CMC (for diazepam treated groups).

Statistical Evaluation of the Data

Data regarding effects of isoproterenol and diazepam on drinking time of deprived rats in "non-conflict" conditions were expressed as cumulative drinking time during 10-minute test sessions and differences were analysed by one-way analysis of variance followed by Dunnett's test. In conflict conditions drug effects on punished and unpunished responses were evaluated on data expressed as cumulative drinking time using Student's t-test for paired comparisons.

RESULTS AND DISCUSSION

Isoproterenol 7.5 and 15 μg/kg prolonged the time spent in drinking of normal deprived rats whereas 30 μg/kg had no significant effect. Similar results were obtained with unpunished responses in the conflict test whereas punished responding was not affected by any dose of isoproterenol. These results clearly show that an increase in primary drive

mechanisms does not necessarily lead to increased punished responses in a conflict test.

In a conflict procedure using untrained rats [1] caffeine was found to increase punished responding at a dose of 50 mg/kg, which did not significantly modify punished or unpunished responses in the present study. Caffeine was found ineffective by Cook and Sepinwall [5] in a lever press conflict procedure.

At doses (2.5 and 5 mg/kg) causing prolonged drinking in normal deprived rats, diazepam did not increase unpunished responding in the conflict test used in the present study. Punished responding, however, was increased by each dose of diazepam (2.5, 5 and 10 mg/kg) though the largest dose of diazepam did not increase drinking in normal deprived rats. These data further show that the effects on punished responses and drinking can be dissociated. In this respect our results are similar to those obtained with various benzodiazepines in operant-conflict procedures [4, 6, 10].

Since isoproterenol and caffeine, unlike benzodiazepines, apparently do not possess any antianxiety activity in man [7], the results indicate that procedures using conditioned punishment of consummatory responses may be more appropriate than those using naive animals for revealing antipunishment activity.

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