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CGS 8216, a Novel Anorectic Agent, Selectively Reduces Saccharin Solution Consumption in the Rat

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KIRKHAM, T. C. AND S. J. COOPER. *CGS 8216, a novel anorectic agent, selectively reduces saccharin solution consumption in the rat.* PHARMACOL BIOCHEM BEHAV **25**(2) 341–345, 1986.—The pyrazoloquinoline CGS 8216, a high-affinity ligand for benzodiazepine. recognition sites, significantly reduced the consumption of a preferred 0.05% sodium saccharin solution in a 30 min two-bottle test. A highly significant effect was detected at 5.0 mg/kg, IP and at higher doses. The consumption of water and 0.6% saline, in two-bottle tests, or of quinine solution and water, in a forced-choice test, was not reliably affected by CGS 8216. The results point to a sensitive and selective intrinsic effect of CGS 8216 on ingestional responses in the rat.

CGS 8216 Fluid intake Quinine Saccharin Salt Sweetness

THE pyrazoloquinoline CGS 8216 binds with high affinity to central-type benzodiazepine receptors [15, 21, 34, 35] and is effective as a benzodiazepine receptor antagonist [1, 4, 12, 28, 30–32]. It also possesses partial benzodiazepine inverse agonist activity [22], and we demonstrated recently that it (together with two other benzodiazepine inverse agonists) produced a dose-dependent reduction in the consumption of a palatable sweetened diet by nondeprived rats [6]. In addition, CGS 8216 reduced the consumption of sweetened milk by nondeprived rats [8]. The anorectic effect of CGS 8216 appears to be mediated by an action at specific benzodiazepine receptors, since it could be reversed by the benzodiazepine antagonist, flumazepil (Ro15-1788) [16]. The effect of CGS 8216 on the consumption of food is present but in an attenuated form in food-deprived and nondeprived rats fed standard laboratory diets [2] (Heath, Kirkham and Cooper, in preparation).

The aim of the present series of studies was to examine the effects of CGS 8216 on the consumption of various solutions by rats. Since the feeding data suggested that sweet taste may be relevant to the action of CGS 8216 on ingestion, we decided to investigate for the first time the effect of CGS 8216 in a two-choice test of saccharin intake and preference. Saccharin was chosen as a non-nutritive sweetener. In further tests, the possible effects of CGS 8216 on the intake of water, saline and a quinine solution were also considered.

EXPERIMENT 1

The aim of the first study was to investigate the effects of CGS 8216 (2.5–20.0 mg/kg, IP) on fluid intake and preference for saccharin solution in water-deprived rats.

METHOD

Animals

The subjects were 23 male hooded rats (General strain) which were bred in the animal laboratory of the Psychology department. They were housed singly in stainless steel cages with continuous access to standard laboratory food pellets (modified Diet 41B, Heygate and Sons, U.K.). They were maintained on a 12 hr light-12 hr dark cycle (lights on at 7 a.m.) and the room temperature was kept constant at 20–21°C. They weighed 340–410 g at the time of testing and had been thoroughly familiarized with handling procedures.

Drug

CGS 8216 (2-phenylpyrazolo-[4,3-c]-quinoline-3-(5H)-one) was ultrasonically dispersed in distilled water to which Tween 80 was added (2 drops to 10 ml). The drug was injected intraperitoneally 15 min before the preference test. Doses (2.5–20.0 mg/kg) were chosen on the basis of previous work [6].

Procedure

Rats were first placed on a schedule of restricted water access and were allocated at random to three groups. Water was removed from cages at 17.00 hr on the day prior to preference testing. At 10.0 hr on each test day rats were placed in test cages (which were identical to home cages) and given 30 min access to two bottles containing the following combinations of fluids: water/water (Group 1, N=8); water/0.05% saccharin sodium solution (Group 2, N=8); water/0.6% sodium chloride solution (Group 3, N=7). Water

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TABLE 1

EFFECTS OF CGS 8216 ON FLUID INTAKE AND PREFERENCE FOR 0.05% SACCHARIN SOLUTION

	CGS 8216 mg/kg						
	0	2.5	5	10	20	F(4, 28)	p
			A. Water vs. water				
Total fluid intake (ml)	12.03 (0.69)	10.13 (0.81)	10.50 (0.80)	10.25 (0.37)	8.88 (1.23)	2.272	NS
% LHS preference	56.20 (4.33)	57.50 (12.67)	58.50 (13.32)	58.75 (12.45)	71.63 (10.08)	0.338	NS
			B. Saccharin vs. water				
Total fluid intake (ml)	19.46 (0.77)	17.88 (1.92)	14.63* (1.65)	13.50† (1.84)	13.25† (0.99)	5.204	0.01
% saccharin preference	82.35 (4.27)	83.13 (5.21)	54.38* (11.72)	65.75 (9.99)	68.25 (14.41)	2.797	0.05
saccharin intake (ml)	16.25 (1.10)	14.75 (1.51)	9.29† (2.22)	9.75† (2.04)	8.88† (1.95)	7.625	.001
water intake (ml)	3.31 (0.97)	3.13 (1.02)	6.13 (1.19)	3.88 (1.33)	4.38 (2.11)	1.482	NS

Results are shown as mean intake (S.E.M.)
N = 8 rats per group.
Levels of significance (Newman-Keuls test): *$p<0.05$; †$p<0.01$.

for drinking and preparation of solutions was ordinary tap water. Fluid intake was determined by weighing bottles and rats were then returned to home cages. Water was restored 30 min later and remained available until 17.00 hr. Food was available at all times except for the 30 min test period. After three days of adaptation to this schedule, rats in Groups 1 and 3 were displaying distinct preferences for the saccharin and saline solutions, respectively. Rats in group 2 showed no obvious side preference. Adaptation continued for a further three days to ensure stability of intake and preference.

Over the following eight days each rat in Groups 1 and 2 received each dose of CGS 8216 (2.5–20.0 mg/kg, IP), with each drug treatment following a vehicle injection on the previous day. Treatment order was counterbalanced within each group, each rat serving as its own control. Rats in Group 3 were similarly tested but over six days at three dose levels of CGS 8216 (5.0–20.0 mg/kg, IP). The position of saccharin and saline bottles were alternated daily to control for position habits of individual animals. Availability of water was as described for the adaptation period.

Data Analysis

Total fluid intake, separate saccharin and saline intakes and percent preferences (flavoured solution intake/total intake × 100) were subjected to individual analyses of variance for each group (1-way ANOVA for repeated measures). Post hoc comparisons were made using the Neuman-Keuls test. Control values were calculated in terms of the mean value for each animal over the three days on which it received a control injection.

RESULTS

As can be seen from Table 1A, CGS 8216 failed to exert any significant effect on the water consumption of rats in Group 1: even the highest dose (20.0 mg/kg, IP) produced only a 24% reduction in intake. Under control conditions rats showed no marked preference for either of the two drinking bottles and drug administration failed to exert any influence on the choice of bottle.

The consumption of water by rats in Group 2 was also unaffected by CGS 8216 administration (Table 1B). There was, however, a significant (25–32%) reduction of total fluid intake following the 5, 10 and 20 mg/kg doses, $F(4,28)=5.204$, $p<0.01$. This resulted from a selective suppression of the intake of saccharin solution: 5, 10 and 20 mg/kg doses of CGS 8216 each reduced intake by 40–45%, $F(4,28)=7.625$, $p<0.001$. Consequently, there was also a reliable, but moderate, drug-induced reduction in saccharin preference ratio, $F(4,28)=2.797$, $p<0.05$. However, it should be noted that although saccharin preference was reduced CGS 8216-treated rats still retained some preference for the solution over water.

Data for Group 3 are summarised in Table 2. Comparison of these data with those for Group 2 shows that, under control conditions, 0.6% saline was preferred over water to the same degree as 0.05% saccharin (approximately 82% in each case). Control intakes of the two solutions were also very similar. However, drug effects on the two solutions were very different: saline intake was not reliably reduced by any dose of CGS 8216. As in the other conditions water intake was unaltered, hence neither saline preference nor total fluid intake were significantly affected by the drug treatments.

DISCUSSION

CGS 8216 (2.5–20.0 mg/kg, IP), did not reduce fluid intake by water-deprived rats indiscriminately. Water intake was not affected, a result which confirms an earlier report [9]. However, our data show for the first time that CGS 8216 significantly reduced the consumption of a preferred sweet solution in a two-bottle preference test. The effect could not be due to a reduction in the intake of preferred solutions *per*

TABLE 2

LACK OF EFFECT OF CGS 8216 ON PREFERENCE FOR 0.6% SALINE

	CGS 8216 mg/kg					
	0	5	10	20	F(3,18)	*p*
Total fluid intake (ml)	22.29 (1.10)	17.43 (0.78)	16.14 (1.96)	18.00 (2.25)	2.401	NS
% saline preference	82.87 (4.43)	90.29 (5.10)	75.57 (9.65)	87.00 (5.08)	1.405	NS
saline intake (ml)	18.99 (1.67)	15.71 (1.09)	13.00 (2.55)	15.71 (2.45)	1.331	NS
water intake (ml)	3.14 (0.82)	1.71 (0.92)	3.14 (0.88)	2.29 (1.02)	0.957	NS

Results are shown as mean intake (S.E.M.).
N = 7 rats per condition.

TABLE 3

EFFECTS OF CGS 8216 ON THE CONSUMPTION OF 0.0005% QUININE SOLUTION OR WATER (FORCED-CHOICE TEST)

	30 Minutes Intake (ml)					
	0	2.5	5	10	20	mg/kg
Water	15.86 (0.99)	15.16 (0.60)	12.51 (1.47)	12.91 (1.47)	12.75 (1.31)	
Quinine	13.96 (0.43)	12.58 (1.43)	11.63 (0.86)	9.76 (1.88)	9.96 (1.71)	

Summary of analysis of variance

Source of variance	SS	*df*	MS	F	*p*
A (liquid)	104.42	1	104.42	(1,14) 3.927	NS
Error AS	372.31	14	26.59		NS
B (dose)	162.75	4	40.69	(4, 16) 1.154	NS
A × B (interaction)	13.28	4	3.32	(4, 16) 0.094	NS
Error B × AS	564.19	16	35.26		
Total	1212.15	79	26.59		

All values are mean (SEM) of 8 rats (2 groups of N = 8)

se. CGS 8216 did not affect the consumption of 0.6% saline, which was matched in terms of preference and intake. A previous report indicates that CGS 8216 did not affect the intake of 1.5% NaCl solution by rats [18]. Hence, CGS 8216 acted selectively to reduce the consumption of the palatable *sweet* solution (Table 1B). The effect was highly significant at a dose of 5.0 mg/kg, which was smaller than the minimally effective dose (10.0 mg/kg) which we previously found to reduce the consumption of a sweetened diet [6]. Saccharin drinking may therefore by particularly sensitive to the suppressant effect of CGS 8216 on ingestion.

EXPERIMENT 2

To examine this idea further, the consumption of water or a non-preferred quinine solution was measured in a one-bottle test following CGS 8216 (2.5–20.0 mg/kg, IP). The aim was to assess further the specificity of the effect of CGS 8216 on fluid consumption.

METHOD

Animals

Sixteen male rats, from the previous study, were used in the second experiment. They were housed as described before.

Procedure

Rats were placed on a schedule of restricted water access. Following 19.5 hr water deprivation, rats were placed in test cages and give 30 min access to a weighed bottle of either tap water or 0.0005% quinine HCl solution (8 rats per group). In a two-bottle test, this concentration was found to be aversive, yielding a mean preference index of 23% (data not shown). Therefore a forced-choice test was used in the present experiment to obtain a relatively high level of quinine consumption, to allow comparisons with the data of Experiment 1. Intake data were analysed by a 2-way (mixed design) ANOVA.

RESULTS AND DISCUSSION

The concentration of quinine HCl in this test had previously been found to be relatively aversive as measured in a two-bottle test (data not shown). However, providing the solution as the sole source of fluid to thirsty rats produced a baseline acceptance level which was comparable with that of water. As can be seen from Table 3, neither the intake of water nor of quinine was reliably reduced by CGS 8216 (2.5–20.0 mg/kg, IP). The drug did not therefore interact with bitterness or enhance any aversive quality of the quinine flavour.

To confirm an effect of CGS 8216 on saccharin solution consumption in a single bottle test, the procedure was repeated using a 0.05% sodium saccharin solution as the test fluid. Five rats were drawn from each of the water and the quinine groups. In this case 10 mg/kg of CGS 8216 reliably reduced 30 min intake from 19.6 (±1.5) to 14.9 (±1.27) ml, $t(9)=3.56, p<0.01$. The values indicated are the mean intake values, ±S.E.M. In subsequent tests, CGS 8216 significantly reduced the consumption of a sweet solution in a single-bottle test in naive animals, indicating that the effect did not depend on prior experience of other flavours (data not shown).

GENERAL DISCUSSION

Our data demonstrate that the pyrazoloquinoline CGS 8216 affected saccharin preference in the rat, and selectively reduced the consumption of the preferred saccharin solution. The intakes of saline, water and a non-preferred quinine solution were not reliably reduced by the CGS 8216 treatments. Hence we have shown that CGS 8216 acts to suppress the intake of a palatable sweetened diet [6], and to reduce the consumption of sweetened milk [8]; in the present report, CGS 8216 selectively affected the intake of saccharin solution. It is possible that CGS 8216 interacts, by some

means, with mechanisms related directly to sweet taste or to incentive motivation associated with sweet taste.

It should be noted that other anorectic compounds do not show this degree of taste-related selectivity. For example, *d*-amphetamine and β-phenylethylamine, besides reducing food intake, also reduce the consumption of water, saccharin and salt solutions [7, 27, 33]. The opiate receptor antagonist naloxone which reduces saccharin preference [5,25], also reduces the intake of water, saline and quinine solutions [10, 13, 23, 24]. Unlike these compounds, therefore, CGS 8216 did not exhibit a general antidipsogenic effect and has been shown previously not to affect water intake or the consumption of 1.5% NaCl solution [9,18]. We are currently investigating the mechanism(s) by which this selective effect may be produced. On possibility is that CGS 8216 imposes a satiety effect, and we are considering the anorectic potential of CGS 8216 in the sham-feeding rat. An effect on intake which interacts with sweetness as a taste factor may provide an explanation, at least in part, for the dose-related reduction in the consumption of a sweetened diet by nondeprived animals [6].

Two other closely-related pyrazoloquinolines, CGS 9895 and CGS 9896 [11,35], appear to have little or no intrinsic effect on the consumption of food [2,14]. Instead, both have been shown to antagonize benzodiazepine-induced hyperphagia [14].

Interactions between benzodiazepine treatments and taste factors have been investigated previously [17,26]. One recent study, using a taste reactivity paradigm, showed that chlordiazepoxide enhanced positive hedonic responses to intraorally-infused solutions [3]. The present data indicate that CGS 8216, a weak benzodiazepine inverse agonist, acted in a converse but selective sense to attenuate the positive response to a preferred saccharin solution. Hence, bidirectional effects on positive responses to gustatory stimuli may be achieved by actions of benzodiazepine agonists and inverse agonists acting at specific benzodiazepine receptors.

Stressors activate the pituitary-adrenal system, and can induce a rise in plasma corticosterone levels. Benzodiazepine receptor inverse agonists like CGS 8216 and the β-carboline compound, FG 7142, increase plasma corticosterone levels in rats that have been habituated to handling (D. Stephens, personal communication), and in rats exposed to a novel environment [29]. Recent data from our laboratory indicate that the anorectic effect of CGS 8216 was identical in adrenalectomized and sham-operated animals (Cooper and Kirkham, unpublished results). Hence, the reduction in ingestional responses could not be attributed to a stress-related rise in plasma corticosterone. The selectivity of the effects of CGS 8216 in the present series of experiments argues further that the anorectic effect of the compound is likely to be unrelated to its proconvulsant or putative 'anxiogenic' effects [19,20].

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