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The Pyrazoloquinoline, CGS 8216, Reduces Sham Feeding in the Rat

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KIRKHAM, T C AND S J COOPER *The pyrazoloquinoline, CGS 8216, reduces sham feeding in the rat* PHARMACOL BIOCHEM BEHAV 26(3) 497–501, 1987 —The pyrazoloquinoline CGS 8216, a benzodiazepine receptor ligand, produced a dose-related (2 5–10 0 mg/kg, IP) attenuation of sham feeding a 30% sucrose solution by rats with open gastric fistulas Ingestion was reduced by over 50% following the largest dose of CGS 8216 in a 60 min test The initiation of sham feeding was not delayed by CGS 8216, but sham feeding was subsequently slowed over an extended test period The suppressant effect of CGS 8216 was reversed by the specific benzodiazepine receptor antagonist Ro15-1788 (20 and 40 mg/kg, IP) Hence the effect of CGS 8216 on sham feeding may be mediated by benzodiazepine receptors, and is consistent therefore with the characterization of CGS 8216 as a benzodiazepine partial inverse agonist In contrast to sham feeding, CGS 8216 (10 0 mg/kg, IP) did not affect sham drinking in 17 hr water-deprived rats The results are discussed in relation to possible benzodiazepine receptor involvement in the neurochemical mediation of food palatability

Benzodiazepines CGS 8216 Palatability Sham feeding Rat Ro15-1788

BENZODIAZEPINE receptor agonists (e g., diazepam, clonazepam, zopiclone, CL 218,872) bring about overconsumption of palatable food in nondeprived rats [4, 6, 7] The drug-induced hyperphagia shows stereospecificity, and can be reversed by the benzodiazepine receptor antagonist Ro15-1788 (flumazepil) [6,8]. The increased ingestional response to palatable food may depend on enhanced positive hedonic reactions produced by benzodiazepine agonists [2] Benzodiazepine receptor *inverse* agonists [3], on the other hand, decrease palatable food consumption in nondeprived rats [5] The pyrazoloquinoline CGS 8216 behaves as a partial inverse agonist [16], and reduces food consumption in nondeprived and deprived animals [1,5] Reductions in sweetened milk consumption produced by CGS 8216, in nondeprived rats, can be reversed by Ro15-1788, indicating that its effect on feeding is benzodiazepine-receptor mediated [9].

The present experiments were designed to provide further information concerning the effects of CGS 8216 on ingestion It has been suggested that sham feeding in the rat (with an open gastric fistula) provides a valuable preparation for analyses of physiological factors that contribute to the maintenance and satiation of feeding responses [24]. The sham-feeding rat exhibits a pronounced satiety deficit [28], and emphasises the importance of oropharyngeal stimulation in maintaining feeding responses. Weingarten and Watson [27] showed that sham feeding sucrose solutions increased as a direct function of sucrose concentration. They suggested that sham feeding serves as a useful procedure for assessing influence of diet palatability on food consumption [27]

Hence, we were interested to establish, for the first time, whether or not CGS 8216 would affect sham feeding in the rat Our plan was to investigate dose-response and time-course relationships for any effect found In addition, it seemed important to establish the reversibility of any effect of CGS 8216 using Ro15-1788, in the light of recent debate on whether or not intrinsic effects of CGS 8216 can be attributed to partial inverse agonist activity at benzodiazepine receptors [5, 9, 12] Since oropharyngeal stimulation is also important in maintaining water drinking [23], a final test determined if CGS 8216 had any effect on sham drinking water

METHOD

Animals

The subjects were thirty-one adult male hooded rats (General strain, bred in this laboratory), and weighed 220–360 g They were housed individually in stainless steel cages, and were maintained under a 12 hr light:12 hr dark cycle (lights on at 07 00 hr). Except for periods of pre-test deprivation and testing, the rats had ad lib access to standard food pellets (Diet 41B, Heygate and Sons, UK) and water. Room temperature was kept constant at 21°C.

Drugs

The drugs used were CGS 8216 (2-phenylpyrazolo [4,3-c] quinoline-3 (5H)-one) which was donated by CIBA-GEIGY Corporation, Summit, NJ, and Ro15-1788 (flumazepil), which was provided by courtesy of Hoffman-La Roche, Basel Both were ultrasonically dispersed in distilled water to which Tween 80 had been added (2 drops to 10 ml) They

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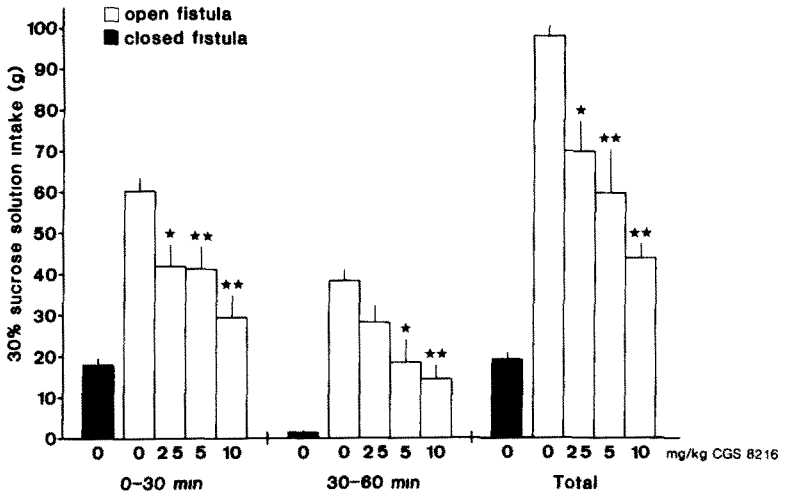

FIG 1 Dose-dependent reductions in sham feeding a 30% sucrose solution produced by CGS 8216 (2 5–10 0 mg/kg, IP) Results are shown as mean±S E M ($n=7$ per condition) for the first 30 min period, second 30 min period, and total 1 hr period, respectively For comparison, data are shown for rats ingesting the sucrose solution following vehicle injection in a closed fistula condition Levels of significance compared with vehicle condition in open fistula condition $*p<0$ 05, $**p<0$ 01 (Dunnett's t-test)

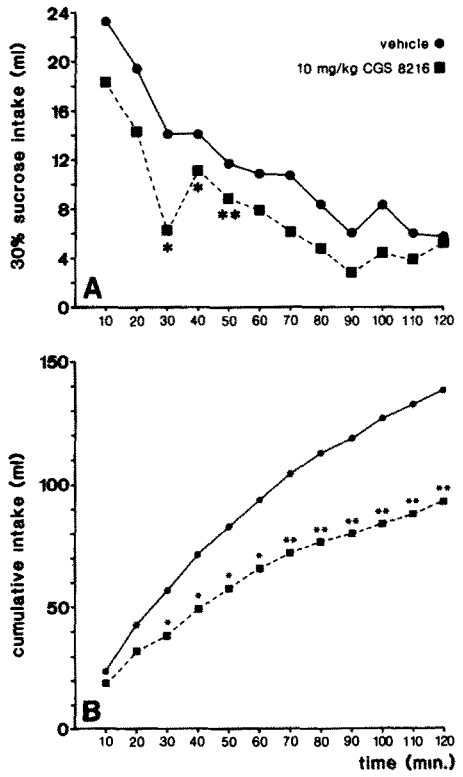

FIG 2 Time-course for the effect of CGS 8216 (10 0 mg/kg, IP) on sham feeding a 30% sucrose in rats with an open gastric fistula Panel A Mean intake (ml) for each consecutive 10 min interval in a 2 hr test Panel B Same data plotted in the form of cumulative intake curves over the 2 hr test N=8 per condition Levels of significance compared with vehicle condition $*p<0$ 05, $**p<0$ 01 (Student's t-test)

were administered IP, either 15 min (CGS 8216) or 5 min (Ro15-1788) prior to tests of ingestion, in a volume of 1 ml/kg body weight Doses were selected on the basis of previous studies [9, 17, 18]

Surgery

The rats were fitted with a chronic gastric fistula using a procedure adapted from [19] Each fistula consisted of a Perspex tube (i d. 6 mm, o.d 8 mm, length 14 mm), which was flanged at each end (diameter 12 mm). A collar of Marlex mesh (Bards Implants, Billerica, MA), 25 mm diameter, was fitted around the fistula shaft and held in place with dental acrylic The fistula was inserted into the greater curvature of the stomach and exteriorised through the abdominal wall and skin A Perspex screw and rubber washer closed the fistula. The surgery was performed while the animals were anesthetised using Sagatal (sodium pentobarbitone) On completion of surgery, rats received an IP injection of a long-lasting analgesic, buprenorphine (Temgesic, 30 μg/kg)

Procedure

In the first experiment, prior to surgery, 7 rats were given 10 min daily access to a 30% (w/v) sucrose solution until stable intake levels were achieved (complete in 4 days) After surgery, rats were given a one-week period of recovery with ad lib access to standard food and water Thereafter they were given daily sham-feeding sessions with a 30% sucrose solution, until intakes stabilised (over a period of 4–5 days) On the last two days, they were given IP injections of isotonic saline

On experimental days, rats were deprived of food from 10 00 hr At 13 45 hr the fistulas were opened, and stomach interiors rinsed with repeated washes of tepid water to remove all traces of food. CGS 8216 (0, 2, 5, 5 0 and 10 0 mg/kg) were then injected After 10 min, stomach interiors were rinsed again to ensure that the fistulas were un-

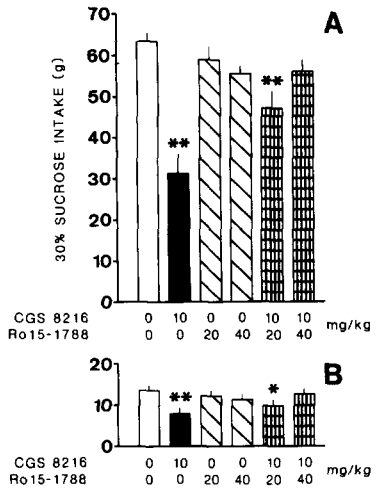

FIG 3 Effects of CGS 8216 (10 0 mg/kg, IP) and Ro15-1788 (20 and 40 mg/kg, IP), alone and in combination, on ingestion of a 30% sucrose solution in (A) sham-feeding rats with an open gastric fistula, (B) intact rats Ro15-1788 reversed the suppressant effect of CGS 8216 on ingestion Intake (g) shown as mean±S E M (n=8 per group) Levels of significance compared with vehicle condition *$p<0$ 05, **$p<0$ 01 (Newman-Keuls test)

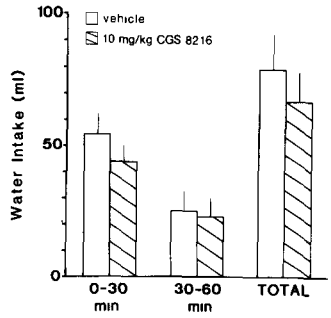

FIG 4 Lack of effect of CGS 8216 on sham drinking water in 17 hr water-deprived rats Results are shown as mean±S E M (n=6 per condition)

obstructed Rats were placed in test cages (identical to home cages) and at 14 00 hr were given access to the 30% sucrose solution in weighed drinking bottles Intakes were then measured gravimetrically after 30 and 60 min At the end of the test, stomachs were rinsed again, fistulas closed and rats returned to their home cages. Drainage was collected in trays placed below cages These were weighed before and after testing and data for individual rats were excluded if the amount collected was less than that consumed.

Each animal received all doses of CGS 8216 according to a randomised schedule, with 48 hr separating successive treatments On intervening days rats were given 30 min sham-feeding sessions

For purposes of comparison, on completion of the sham-feeding tests, 60 min intake was again tested following vehicle injection but with fistula screws in place so that normal satiation could occur Prior to testing, fistulas were opened, stomachs emptied and fistulas resealed.

For the second experiment, 8 additional rats were fistulated, and familiarised with the 30% sucrose solution and the sham-feeding procedure, as described for the first experiment. Following the establishment of stable sham-feeding baselines, rats were injected with either 10 mg/kg of CGS 8216 or its vehicle The deprivation, pre- and post-test procedures followed those used in the first experiment The order of injection was counterbalanced. four rats received a vehicle injection on day 1, and four received CGS 8216 The injections were reversed on day 2, which was separated from the day 1 test by 48 hr Sucrose intake was measured volumetrically at 10 min intervals over a 2 hr period, the 30% sucrose was presented in 1 ml calibrated drinking tubes

For the third experiment, 8 further rats were fistulated and trained as described above Fifteen minutes before testing rats were injected with either vehicle or 10 mg/kg of CGS 8216 Ten minutes later animals received a second injection of vehicle, 20 or 40 mg/kg of Ro15-1788 Rats were then placed in test cages and after 5 min were given access to weighed drinking bottles containing 30% sucrose solution. After 30 min bottles were reweighed, fistulas closed and the rats returned to their home cages Each animal received all combinations of treatments according to a randomised schedule Successive treatments were separated by 48 hr, with a 30 min sham-feeding session being given on each intervening non-test day An additional group of 8 non-operated rats were also tested under the same drug-combination conditions, after they had attained stable levels of sucrose ingestion in daily 10 min tests

In the final experiment, six animals, which had previously been used in the first experiment, were adapted to a schedule of limited access to water. Water was made available from 10.00–17 00 hr (during the light period). After two days adaptation, they were then given daily 30 min sham-drinking sessions, beginning at 10.00 hr. Fistulas were resealed, and 30 min after the end of each session, water was restored to the home cage Following stabilisation of sham-drinking levels (4 days), the rats were injected with either 10 mg/kg of CGS 8216 or its vehicle 15 min before water was presented in weighed drinking bottles Water intake in the sham-drinking test was measured gavimetrically after 30 and 60 min. Preparation of the animals pre- and post-testing was as described for the sham-feeding experiments The two treatments were separated by a 48 hr interval and the order of injections was counterbalanced across animals. The effects of CGS 8216 on sham drinking were analysed using analysis of variance, followed by tests for multiple comparisons, and by Student's t-test

RESULTS

Sham Feeding CGS 8216 Dose-Response

Figure 1 confirms that sham-feeding rats ingested large quantities of the highly palatable 30% sucrose solution Control levels of intake were 60 0±3.3 g (mean±SEM) at 30 min, and rising to 98 5±2.6 g by the completion of the 60 min period of testing. By way of comparison, in the closed fistula condition, rats consumed only 18 0±1.3 g in the first 30 min, and had virtually ceased to ingest the sucrose in the second 30 min period. In the sham-feeding condition, therefore, the rats consumed about five times as much sucrose solution compared with the closed fistula condition, in which gastric contents passed normally into the duodenum.

The pyrazoloquinoline CGS 8216 produced a highly significant, dose-related reduction in the total 60 min intake of sucrose, $F(3,18)=12.21$, $p<0.001$ A significant decrease in sham feeding occurred at 2.5 mg/kg of CGS 8216, and the

highest dose reduced 60 min intake by 55% A complete abolition of sham feeding did not occur since intake after 10.0 mg/kg of CGS 8216 was still greater than 60 min intake in the closed fistula condition. The effect of CGS 8216 was evident within the first half of the sham-feeding test, $F(3,18)=7\ 01$, $p<0.01$, and all doses significantly attenuated ingestion. At 10.0 mg/kg, CGS 8216 produced a 51% reduction in intake. During the 30–60 min period, CGS 8216 continued to reduce sham feeding significantly, $F(3,18)=6\ 18$, $p<0.01$, and there was a 62% reduction at the highest dose

Sham Feeding CGS 8216 Time-Course

The pattern of sham feeding over 120 min following either CGS 8216 (10.0 mg/kg) or vehicle is shown in Fig 2. When administered CGS 8216, rats approached the sucrose solution and began ingesting it as avidly as under control conditions (latencies to initiate feeding were negligible in either case) Initially, intake of the sucrose solution did not differ significantly between drug and control conditions When treated with CGS 8216, the rats showed a slightly lower level of intake over the first 20 min period, but the difference was not significant Beyond 20 min, however, CGS 8216 did produce significant reductions in sham feeding. As Fig. 2B indicates, cumulative ingestion of the sucrose solution reached about 140 ml by the end of the 2 hr test, under control conditions. The pyrazoloquinoline attenuated, but did not abolish, sham feeding. Ingestion reached about 90 ml following administration of CGS 8216 Hence, CGS 8216 had a delayed effect, which resulted in a slowing but not complete cessation of sham feeding.

Reversal of CGS 8216 Effect by Ro15-1788

In sham-feeding rats CGS 8216 (10 0 mg/kg) produced a significant 50% reduction in ingestion of the sucrose solution in a 30 min test (Fig. 3A). Neither dose of Ro15-1788 (20 or 40 mg/kg) had any significant effect on sham feeding when it was administered alone. However, given in combination with CGS 8216, Ro15-1788 at 20 mg/kg attenuated, and at 40 mg/kg, almost completely reversed the suppression of sham feeding by the pyrazoloquinoline.

A similar pattern of results was obtained in non-operated animals (Fig. 3B). By itself, CGS 8216 (10 0 mg/kg) significantly reduced ingestion of the 30% sucrose solution. Ro15-1788 by itself had no effect, but at 40 mg/kg it antagonised the suppressant effect of CGS 8216

Sham Drinking and CGS 8216

Under the conditions of testing, rats were sham drinking substantial amounts of water (Fig 4). The level of consumption reached 54.1 ± 7.8 ml by 30 min, rising to $79\ 5\pm13\ 2$ ml at the end of the 60 min test. At 10 mg/kg, CGS 8216 had no significant effect on sham drinking.

DISCUSSION

Rats, tested with the gastric fistula open, consumed the 30% sucrose solution considerably in excess of that consumed when they ingested normally in the closed fistula condition The pyrazoloquinoline CGS 8216 not only reduced normal ingestion (Fig. 3B), but, more significantly, produced a dose-dependent reduction in sham feeding (Fig. 1). CGS 8216 did not completely block sham feeding, in the range of doses that were tested, but did produce in excess of 50% reduction at the highest dose (10.0 mg/kg). Since this degree of suppression has been found in intact animals consuming palatable foods and fluids [9, 17, 18], we suggest that much, if not all, of the effect of CGS 8216 on food ingestion depends on preabsorptional consequences of normal ingestion

Data from the present series of experiments indicate that it is unlikely that CGS 8216 attenuated sham feeding because of nonspecific behavioural impairment or interference Initiation of sham feeding was not affected and animals began ingestion with great avidity following administration of CGS 8216 Importantly, sham drinking of water remained unaffected by CGS 8216 at a dose which produced a substantial decrement in sham feeding (cf [10, 17, 18]). Effects for CGS 8216 on sham feeding may therefore be differentiated from either its proconvulsant activity or putative proconflict effects [11,21] In addition, it has been reported that CGS 8216 at 3 mg/kg did not have aversive properties as determined in a place preference conditioning paradigm [26] The characteristic effect of CGS 8216 on sham feeding was to retard the rate of ingestion to produce a reduction in the slope of the cumulative intake curve (Fig 2B). A very similar effect can be obtained by reducing the sucrose concentration in the sham-feeding test [15]

Hyperphagic effects of benzodiazepine receptor agonists are reversed by the antagonist Ro15-1788 [6, 14, 20]. The present data show that Ro15-1788 also antagonized the reduction in sham feeding produced by CGS 8216 This result agrees closely with our earlier finding that Ro15-1788 antagonised the effect of CGS 8216 on sweetened milk consumption in nondeprived, intact rats [9]. Both sets of data support the view that effects of CGS 8216 on ingestion are mediated by benzodiazepine receptors It is interesting to note that, by itself, Ro15-1788 had no effect on sham feeding, even though in many experimental situations it exhibits some intrinsic activity [13]

Studies using quantitative autoradiography show that the distribution of [^{3}H] CGS 8216 binding sites in rat brains is very similar to that of [^{3}H] flunitrazepam sites [25] We suggest, therefore, that CGS 8216 acts *centrally* to reduce the palatability of ingested food Previously it has been shown that CGS 8216 reduced consumption of sweetened mash, sweetened milk and a highly palatable saccharin-glucose solution in nondeprived rats [5, 9, 18] It also attenuated saccharin preference in a two-choice test [17] The present results are consistent with the possibility that CGS 8216 attenuates the hedonically-positive effects of oropharyngeal stimulation They imply that central benzodiazepine receptors may be involved in the mediation of palatability responses to food ingestion, in agreement with results from a study using a taste reactivity paradigm [2] In further support of the view, we have recently found that midazolam, a benzodiazepine receptor agonist, significantly increased sham feeding in the rat (Cooper, van der Hoek and Kirkham, unpublished data) Dopaminergic and endorphinergic mechanisms have also been implicated in the maintenance of sham feeding in the rat [15,22] Taken together, these reports and our present data provide pharmacological data of immediate relevance to the neurochemical mediation of food palatability in the maintenance of feeding

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