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Differential Effects of CGS 8216 and Naltrexone on Ingestional Behaviour

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KIRKHAM, T. C., D. J. BARBER, R. W. HEATH AND S. J. COOPER. *Differential effects of CGS 8216 and naltrexone on ingestional behaviour.* PHARMACOL BIOCHEM BEHAV **26**(1) 145–151, 1987.—Effects of the pyrazoloquinoline CGS 8216 (a partial benzodiazepine receptor inverse agonist) and the opiate antagonist, naltrexone, were compared in several tests of ingestion in non-deprived and deprived male rats. Both naltrexone (0.1–10.0 mg/kg, SC) and CGS 8216 (1.25–10.0 mg/kg, IP) significantly reduced the consumption of a highly palatable saccharin-glucose solution by non-deprived rats. Both compounds were also effective in reducing, dose-dependently, the intake of palatable sweet or oily mash by non-deprived animals. Hence, naltrexone and CGS 8216 attenuated palatability-induced ingestional responses, and sweet taste was not necessary for this effect to occur. The two drugs also reduced the intake of the saccharin-glucose solution in food-deprived rats, but their effects diverged in water-deprived animals. CGS 8216 had relatively little effect in the thirsty animals, whereas the effect of naltrexone was enhanced. This difference was underscored in a final test of deprivation-induced consumption of water. Naltrexone reduced the drinking, but CGS 8216 had no effect. Taken together, these data indicate that CGS 8216 was more selective in its effects on ingestion.

CGS 8216 Food- and water-deprivation Naltrexone Palatability Saccharin-glucose Thirst Rat

THE pyrazoloquinoline CGS 8216 (2-phenylpyrazolo-[4,3-c] -quinoline-3(5H)-one) binds with very high affinity to central benzodiazepine receptors [8, 21, 28, 48, 50]. It was described as a benzodiazepine receptor antagonist [7, 21, 50], and reverses the effects of benzodiazepines in behavioural and electrophysiological experiments (e.g., [2, 7, 8, 27, 33, 34, 36, 41, 43, 44]). The hyperphagic effect of benzodiazepines, which is reversed by the imidazobenzodiazepine flumazepil (Ro15-1788) in rats, rabbits and rhesus monkeys [15, 18, 19, 26, 32], is also effectively antagonized by CGS 8216 [18,19]. Recently it has been shown that some benzodiazepine receptor ligands have effects which are opposite to those of the 1,4-benzodiazepines, and these novel compounds have been described as "inverse agonists" [9, 30, 37]. On the basis of biochemical, pharmacological and behavioural criteria, CGS 8216 can be described as a partial benzodiazepine inverse agonist [24, 25, 30, 48]. It should therefore reduce the consumption of food, in contrast to the hyperphagic effect of benzodiazepines. Recent data confirm its anorectic effect in food-deprived rats consuming standard laboratory food [6], and in non-deprived animals eating a palatable sweet mash [14], or consuming sweetened milk [22]. The anorectic effect of CGS 8216 appears to be benzodiazepine receptor-mediated, since it can be antagonized by flumazepil [22]. It is interesting to note that the structurally-related pyrazoloquinolines, CGS 9895 and CGS 9896 [3–5, 8], have little or no intrinsic effect on feeding responses in the rat [6, 15, 19, 39], but do antagonize benzodiazepine-induced hyperphagia [19]. Hence the anorectic effect of CGS 8216 depends on a minor modification to the pyrazoloquinoline chemical structure.

The general aim of the present series of experiments was to compare and contrast the effects of CGS 8216 with those of the opiate receptor antagonist, naltrexone, in several tests of feeding and drinking behaviour. Naltrexone, like other opiate receptor antagonists, reduces deprivation-induced as well as palatability-induced feeding and drinking responses in the rat [1, 10–13, 16, 17, 29, 31, 38]. An opiate antagonist was considered to be an appropriate comparison drug, since compounds like amphetamine, fenfluramine and other phenylethylamine derivatives, exhibit steep dose-responses curves and, in large doses, completely suppress feeding responses [20,38]. On the other hand, opiate antagonists generally produce shallow dose-response curves, and do not achieve complete suppression of ingestional responses [38]. On the basis of currently available data, CGS 8216 tends to produce a shallow dose-response relationship, e.g., in food-deprived rats eating standard laboratory chow [6]. The emphasis of the present work was placed, therefore, on comparisons between CGS 8216 and naltrexone to assess the degree of similarity between their effects for several kinds of ingestional response.

Three tests of ingestion were chosen. In the first, a highly palatable saccharin-glucose solution, which is consumed in large amounts by non-deprived animals [46], was used. Ingestion that is motivated by incentive factors in need-free

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animals was investigated using this paradigm. It has previously been reported that naloxone and a dopamine antagonist, pimozide, reduced the consumption of a saccharin-glucose solution [42,49]. The data were interpreted in terms of inhibition of the incentive to drink the highly palatable solution. Since deprivation state may interact with palatability in determining the effects of drug treatments, three groups of animals were tested: non-deprived, food-deprived and water-deprived, respectively. The second test continued the investigation of palatability-induced ingestion. The consumptions of two palatable mashes were compared; one was sweetened, while the second was made palatable by adding vegetable oil. Hence, it was possible to determine if the effects of drug treatments on consumption in need-free animals depends on sweet taste as such, or whether the drugs would have more general effects on palatability-induced feeding. The final test investigated the effects of naltrexone and CGS 8216 on drinking motivated by water-deprivation, in an attempt to assess the specificity of their effects on ingestional responses. It also allowed a comparison between drinking in response to water-deprivation, and drinking in response to the incentive of a palatable taste (cf. [42]).

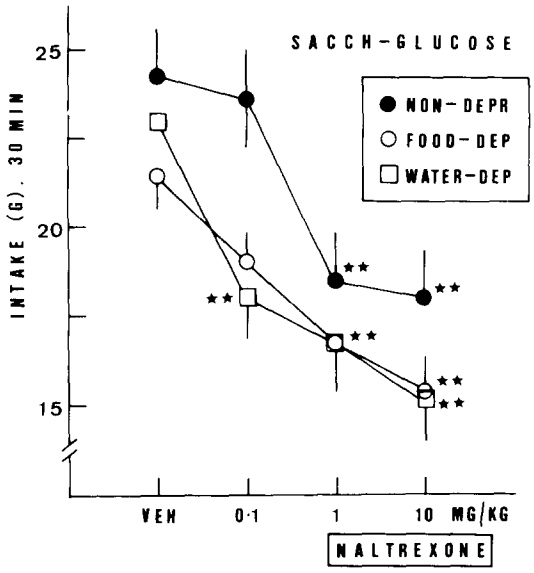

FIG. 1. Effects of naltrexone (0.1–10.0 mg/kg, SC) on the consumption of a palatable saccharin-glucose solution in male non-deprived (●), food-deprived (○), and water-deprived (□) rats. Results are shown as mean intake (g) in a 30 min test. S.E.M.'s are indicated by vertical lines (some omitted for clarity). N=10 per group. Levels of significance: ★$p<0.05$; ★★$p<0.01$ (Dunnett's t-test).

METHOD

Animals

The subjects were 60 adult 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 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 180–210 g at the start of the study, and had been thoroughly familiarized with handling.

Drugs

Naltrexone hydrochloride (generously provided by DuPont de Nemours, Glenolden, PA) was dissolved in isotonic saline. It was injected subcutaneously 20 min before the ingestional tests. Doses (0.1–10.0 mg/kg) refer to the salt, and were chosen on the basis of published work [11,16]. CGS 8216 (generously provided by CIBA-GEIGY, Summit, NJ) was ultrasonically dispersed in distilled water to which Tween 80 was added (2 drops in 10 ml). It was injected intraperitoneally 20 min before the tests, and the doses (1.25–10.0 mg/kg) were chosen on the basis of previous work [6,14].

Procedure

Rats were allocated at random to six groups (N=10 per group), and were adapted as follows. The first three groups consisted of the following: (1) non-deprived group with ad lib access to food and water; (2) animals placed on a 22 hr daily food-deprivation schedule: water was available ad lib and the standard food pellets were available for 2 hr each day; (3) animals placed on a 22 hr daily water-deprivation schedule: food was available ad lib and water was provided for 2 hr each day. At the end of the deprivation periods, animals were given access to a 5% *d*-glucose + 0.2% sodium saccharin solution for 60 min in the home-cage. This was presented in an inverted tube, equipped with a stainless-steel spout, which was clipped to the front of the home-cage. Each tube was weighed to the nearest 0.1 g on a Sartorius balance, before and after the 60 min period, and the amount consumed was calculated by subtraction. Non-deprived animals were presented with the saccharin-glucose solution at the same time as the deprivation-condition groups.

The fourth and fifth groups were given 60 min daily access to a palatable sweet mash and a palatable oily mash, respectively. The animals were not deprived of either food or water prior to presentation of the mashes. About 40–50 g of the freshly prepared diet was spooned into a clean Perspex petri dish, and was positioned inside each home cage. The sweet diet was made to the following formula: 50 ml Nestle's brand sweetened condensed milk, 150 ml ground food (rat and mouse expanded ground diet No. 1, Special Diet Services Ltd., Essex), and 200 ml water, and has been used in previous studies [14, 15, 19]. The non-sweet diet was constituted to match approximately the sweet mash both in consistency and control levels of intake and was made as follows: 10 ml vegetable oil (VG brand), 150 ml ground food, and 240 ml water. Both mashes were prepared and mixed thoroughly immediately before use. Consumption was measured by successive weighings to an accuracy of 0.1 g. Care was taken to collect any food spillage, and to make appropriate corrections to the weighings.

The sixth group were adapted to a daily 22 hr water-deprivation schedule. They were given 60 min access to water in an inverted tube clipped to the front of the home-cage, followed by a further 60 min access to an automatic watering-system. Intake during the first 60 min was measured by successive weighing of the drinking tubes to the nearest 0.1 g. Standard food pellets were available ad lib.

All six groups were adapted to these procedures for a period of 14 days before drug-testing began. Animals were weighed daily before the 60 min ingestion tests, and all testing took place in the light (10 a.m.–4 p.m.). By the end of this

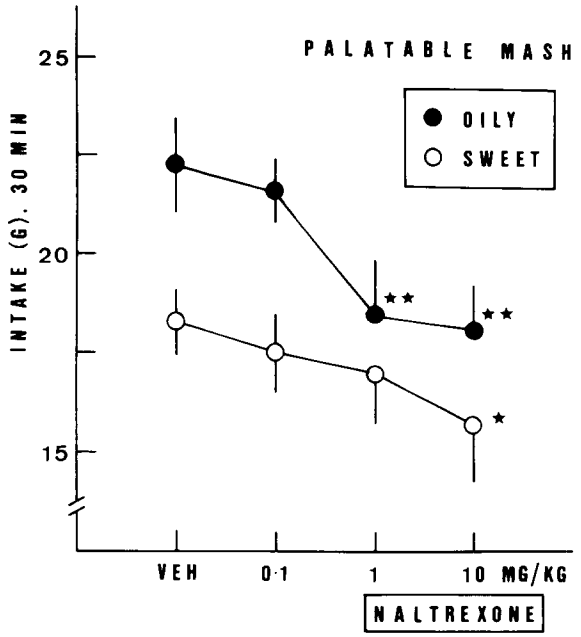

FIG. 2. Effects of naltrexone (0.1–10.0 mg/kg, SC) on the consumption of a palatable oily mash (●), or a palatable sweet mash (○), by non-deprived male rats. See Fig. 1 legend for other details.

TABLE 1

ANTIDIPSOGENIC EFFECT OF NALTREXONE, BUT LACK OF EFFECT ON CGS 8216, IN REHYDRATING MALE RATS

Naltrexone (mg/kg, SC)				
0	0.1		1.0	10.0
19.3 ±0.8	18.3 ±1.2		15.2* ±0.9	13.5* ±0.7
CGS 8216 (mg/kg, IP)				
0	1.25	2.5	5.0	10.0
18.5 ±0.9	18.3 ±0.6	16.7 ±0.9	17.3 ±0.9	17.8 ±0.7

Results are shown as mean ± SEM for water intake (g). N = 10 per group.

Levels of significance: *$p<0.01$ (Dunnett's t-test).

Thirty min test of water consumption.

adaptation period, latencies to begin ingestion were at an absolute minimum in all animmals.

Experiments were conducted with CGS 8216 first. Each rat was tested on five occasions following administration, respectively, of 1.25, 2.5, 5.0 and 10 mg/kg of CGS 8216 and of vehicle, in counterbalanced mixed order. Tests were conducted over a period of two weeks, with at least 48 hr between successive injections. After a break of 5 days, in which the animals were treated under the pre-drug adaptation conditions, each rat was tested on four occasions following administration , respectively, of 0.1, 1.0 and 10 mg/kg of naltrexone and of vehicle, in counterbalanced mixed order. There were 48 hr intervals between successive injections. For the drug experiments, the time of access to the saccharin-glucose solution, sweet and oily mashes, and water, was reduced to 30 min, since most consumption occurred within the first 30 min. During the subsequent 90 min period, standard food pellets and water were available to the animals, before the start of the next deprivation period in the deprivation groups. Non-deprived groups, of course, remained on ad lib food and water. All intake data, therefore, are reported in terms of consumption (g) over 30 min.

The intake data were analysed using either a two-way (mixed design) analysis of variance, or a one-way ANOVA for repeated measures. The level of significance of differences between intake under vehicle conditions and drug conditions were evaluated using Dunnett's t-test [47]. Since naltrexone was used as a reference compound, its data are presented before those of CGS 8216.

RESULTS AND DISCUSSION

Experiment 1. Effects of Naltrexone

Saccharin-glucose solution consumption. Naltrexone (0.1–10 mg/kg SC) significantly reduced the consumption of the highly palatable saccharin-glucose solution, $F(3,81)=33.1$, $p<0.001$. There were differences between the three groups, $F(2,27)=3.61$, $p<0.05$; non-deprived rats consumed 24.2±1.5 g (mean±SEM) following injection of the vehicle, while food-deprived and water-deprived animals consumed slightly less, 21.4±0.9 and 22.9±1.3 g, respectively. The drug × group interaction term was not significant, $F(6,81)=1.27$, N.S., and naltrexone signficantly reduced consumption in each of the three groups (Fig. 1). The water-deprivation animals were more sensitive than the others, and showed a significant reduction in intake at 0.1 mg/kg of naltrexone. At the higher dose of 1.0 mg/kg, naltrexone also significantly reduced consumption of the palatable solution in non-deprived and food-deprived animals. Maximum percentage reductions produced by naltrexone were 25.7%, 28.5%, and 34%, for non-deprived, food-, and water-deprived groups, respectively.

Sweet or oily mash consumption. Naltrexone produced significant reductions in the consumption of palatable mashes by non-deprived rats, $F(3,54)=6.10$, $p<0.005$. Slightly more oily mash was consumed than the sweet mash, $F(1,18)=5.74$, $p<0.05$, but there was no significant drug dose × group interaction, $F(3,54)=1.13$, N.S. Naltrexone was effective at 1.0 mg/kg in reducing consumption of the oily mash, but its effect on the intake of the sweetened mash only reached significance ($p<0.05$) at 10 mg/kg (Fig. 2).

Consumption of water. Naltrexone significantly reduced water intake in rehydrating animals, $F(3,36)=16.88$, $p<0.001$, and achieved effects which were significant at 1.0 and 10.0 mg/kg (Table 1). The maximum percentage reduction was 27.9%. An additional analysis carried out on the data for the water-deprived animals drinking water, and for water-, and non-deprived animals drinking the saccharin-glucose solution emphasized the significant drug effect, $F(3,81)=37.3$, $p<0.001$, but the drug dose × group interaction was not significant, $F(6,81)=1.67$, N.S.

Taken together, the results show that naltrexone reduced consumption in each of the feeding and drinking tests, although there were variations in the sensitivity of each test to the effect of naltrexone. The least sensitive test of ingestion occurred in the case of the non-deprived animals consuming

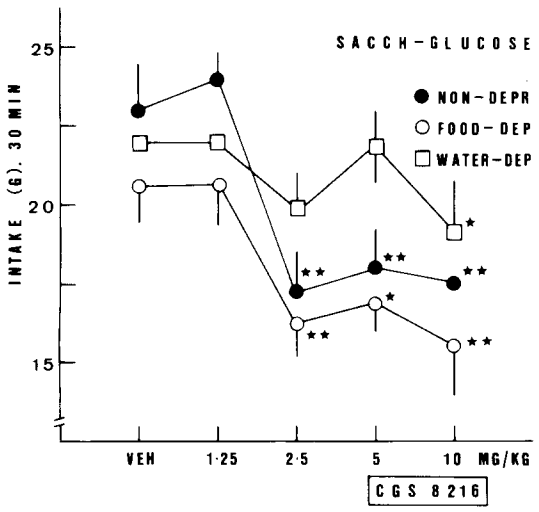

FIG. 3. Effects of CGS 8216 (1.25–10.0 mg/kg, IP) on the consumption of a palatable saccharin-glucose solution in male non-deprived (●), food-deprived (○), and water-deprived (□) rats. See Fig. 1 legend for other details.

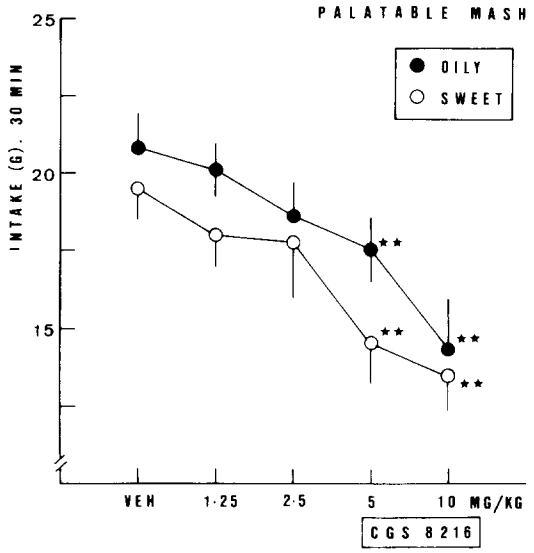

FIG. 4. Effects of CGS 8216 (1.25–10.0 mg/kg, IP) on the consumption of a palatable oily mash (●), or a palatable sweet mash (○), by non-deprived male rats. See Fig. 1 legend for other details.

the sweetened mash (a significant effect at 10.0 mg/kg), while the most sensitive were the water-deprived animals drinking the saccharin-glucose solution (a significant effect at 0.1 mg/kg). All other groups were intermediate, with a minimally-effective dose of 1.0 mg/kg. Sweet taste was evidently not a necessary factor in the anorectic effect of naltrexone, since the consumption of palatable oily mash was also significantly reduced. Food-deprivation did not counteract the effect of naltrexone on the ingestion of the saccharin-glucose solution, although an earlier report indicated that food-deprivation attenuated an anorectic effect of naloxone [40]. Indeed, water-deprivation appeared to make the animals relatively more sensitive to the effect of naltrexone. It has also been previously reported that naloxone was more effective in reducing the intake of sweet solution by non-deprived rats than the water intake of water-deprived rats [42]. The present data, using naltrexone, failed to support the distinction. In summary, naltrexone significantly attenuated consumption of a highly-palatable saccharin-glucose solution, of oily and sweet mashes (in non-deprived animals), and of water by rehydrating animals. Water-, but not food-, deprivation made saccharin-glucose consumption more sensitive to naltrexone's attenuating effect.

Experiment 2. Effects of CGS 8216

Saccharin-glucose solution consumption. CGS 8216 (1.25–10 mg/kg, IP) also significantly reduced the consumption of the palatable saccharin-glucose solution, $F(4,108)=17.17$, $p<0.001$. There were slight differences between the three groups, $F(2,27)=3.38$, $p<0.05$; non-deprived rats consumed 23.0±1.6 g following vehicle, and the food-deprived and water-deprived animals consumed slightly less, 20.5±1.1 and 22.1±1.8 g, respectively. The drug dose × group interaction was not significant, $F(8,108)=1.61$, N.S. As Fig. 3 indicates, CGS 8216 reduced the consumption of the palatable saccharin-glucose solution at a minimally-effective dose of 2.5 mg/kg, in both non-deprived and food-deprived groups. In water-deprived animals, however, CGS 8216 achieved only a marginal effect at 10 mg/kg. Maximum percentage reductions produced by CGS 8216 were 33.9%, 30.6%, and 13%, for non-deprived, food-, and water-deprived groups, respectively (note the reverse in rank order compared with that of naltrexone). Hence, water-deprived animals were *least* sensitive to the attenuating effect of CGS 8216 on consumption.

Sweet or oily mash consumption. CGS 8216 produced dose-related reductions in the consumption of the palatable mashes by non-deprived rats, $F(4,72)=15.58$, $p<0.001$. There was not a significant group effect, $F(1,18)=1.97$, N.S., or drug dose × group interaction ($F<1.0$). Hence, CGS 8216 had equivalent effects on the intake of the sweet or oily mashes, producing significant reductions in both groups at 5 and 10 mg/kg (Fig. 4).

Consumption of water. CGS 8216 had no significant effect on the consumption of water in water-deprived animals ($F<1.0$) (Table 1).

These data extend previous observations that CGS 8216 significantly reduced the consumption of dry food by food-deprived rats [6], and the consumption of a sweet mash, or sweetened milk, by non-deprived animals [14,22]. Water-deprived animals appeared to be insensitive to the attenuating effect of CGS 8216 on ingestion, since the consumption of water was unaffected and that of the highly-palatable saccharin-glucose solution was only slightly affected in rehydrating animals. A similar insensitivity has been observed in water-deprived animals drinking hypertonic saline [23]. It would be particularly interesting to determine if CGS 8216 is also ineffective in reducing water intake in response to other dipsogenic stimuli (e.g., angiotensin II, isoproterenol, hypertonic saline, polyethylene glycol). The present observations in water-deprived animals suggest that CGS 8216 does not

TABLE 2

COMPARISONS BETWEEN THE EFFECTS OF NALTREXONE AND CGS 8216 ON INGESTIONAL RESPONSES IN RATS

		Feeding[a]	Feeding		Drinking			Drinking
		F-D[b]	N-D		N-D	F-D	W-D	W-D
Drug	Action	Dry Pellets[c]	Sweet Mash	Oily Mash	Sacch-Glucose	Sacch-Glucose	Sacch-Glucose	Water
Naltrexone	opiate receptor antagonist	reduced* intake	slight effect on intake	reduced intake	reduced intake	reduced intake	enhanced reduction in intake	reduced intake
CGS 8216	benzodiazepine receptor inverse agonist	reduced† intake	reduced intake	reduced intake	reduced intake	reduced intake	little effect on intake	no effect

F-D (food-deprived); N-D (non-deprived); W-D (water-deprived).
[a]Response required for ingestion.
[b]Presence or absence of deprivation conditions.
[c]Type of commodity ingested.
*Data from [38]; †data from [6].

block drinking responses aroused by thirst. In contrast, it is effective in attenuating drinking responses motivated by the palatability of sweet taste as the data for non-deprived animals consuming the saccharin-glucose solution clearly demonstrated. Furthermore, this effect was not restricted to either drinking responses, or to ingestion motivated by sweet taste. Eating responses aroused by highly palatable sweet and oily mashes were equally affected by CGS 8216. The pyrazoloquinoline, therefore, attenuated both eating and drinking responses aroused by incentive factors in non-deprived rats.

GENERAL DISCUSSION

Previously, both CGS 8216 and naltrexone have been shown to attenuate the consumption of dry laboratory diet in food-deprived rats [6,38]. However, naltrexone also reduced the consumption of water by water-deprived rats [11,16], whereas CGS 8216 did not (Table 2). Hence, the two drugs share the effect of reducing feeding in response to motivation aroused by need, but were clearly distinguished in a test of drinking in response to dehydration. It should be noted that naltrexone does not reduce ingestional responses indiscriminately, since it, together with other opiate antagonists, failed to reduce schedule-induced polydipsia in rats [10,16]. Rats can continue, therefore, to generate the motor acts required in drinking following treatments with opiate antagonists.

When feeding and drinking responses motivated by palatability are considered, the effects of CGS 8216 and naltrexone were generally quite similar. The consumption of the saccharin-glucose solution, and of the sweet and oily palatable mashes were significantly reduced by both drugs. Sweet taste was clearly not a necessary requirement for either CGS 8216 or naltrexone to attenuate palatability-induced ingestion, and this represents a novel and important finding for both drugs [13, 14, 17, 29, 31]. A further point of contrast occurred, however, in the case of water-deprived rats consuming the saccharin-glucose solution. These animals were more sensitive to naltrexone's effect than either non-deprived or food-deprived rats, when minimally-effective doses are considered. In contrast, this group was less sensitive to the attenuating effect of CGS 8216 on consumption.

The data summarized in Table 2 suggest that naltrexone attenuated feeding and drinking responses motivated by either deprivation or palatability. CGS 8216 appears to act, in some respects like naltrexone, but attenuated ingestional behaviour more selectively. Thirst-aroused drinking was not reduced by CGS 8216, and indeed, the state of water-deprivation appeared to antagonize the effect of CGS 8216 in animals consuming the saccharin-glucose solution. The reason for this antagonism is unclear, but it could arise if thirsty animals consume the saccharin-glucose solution not for the sweet taste, but for the rehydrating effect of the ingested solution. In that event, the effect of CGS 8216 would be expected to be negligible. An alternative view arises from a possible inhibitory effect of water deprivation on the motivation to feed [45], and this may interact in a general way with the effect of CGS 8216 on the ingestion of food. However, recent data of ours suggest that water-deprivation does not block the effect of CGS 8216 in animals consuming dry food (Heath, Kirkham and Cooper, unpublished data). Therefore, the former appears to be the more likely explanation.

Other experiments indicate that the intrinsic effects of CGS 8216 include a proconvulsant or a putative anxiogenic effect [24,25]. There are several arguments, however, which suggest that the effects of CGS 8216 on ingestion are not merely secondary to these effects. First, the effect of CGS 8216 on ingestion was selective, and intake of hypertonic saline was unaffected by it (cf. [23]). Second, the anorectic effect of CGS 8216 was found to be identical in adrenalectomized and sham-operated rats (Cooper and Kirkham, submitted for publication). Although CGS 8216 causes an elevation of plasma corticosterone, which may be stress-related [35], its anorectic effect must be independent of this rise. Third, CGS 8216 at 3 mg/kg did not have aversive properties as determined in a place preference conditioning paradigm [44], although at 2.5 mg/kg it produced a significant decrease in saccharin-glucose consumption (Fig. 3). Fourth, CGS 8216 does not affect the latency to begin feeding, and its effect on consumption is proportionately greater towards the end of a meal compared with its effect at the beginning [22]. In addition, it is interesting that in trials conducted in human volunteers, oral doses of CGS 8216 (1–650 mg) did not

produce changes of mood, or anxiogenic effects (Bieck, personal communication). For these reasons, it seems probable that effects of CGS 8216 on food and fluid consumption may be more directly related to processes governing ingestion. Since benzodiazepine agonists enhance ingestive behaviour [14,15], the data for CGS 8216 in the present report are consistent with its action as a partial inverse agonist at least in relation to feeding.

In summary, CGS 8216 and naltrexone were similar, in certain important respects, in that both attenuated the consumption of palatable mashes in non-deprived animals, and both reduced the consumption of a palatable saccharin-glucose solution. However, naltrexone was also effective in lowering the ingestion of water by thirsty rats, while CGS 8216 did not affect deprivation-induced drinking. We suggest that the effects of CGS 8216 on ingestional responses, at least in animals fully familiarized with test conditions, are not secondary to a putative anxiogenic effect.

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