*Pharmacology Biochemistry & Behavior*, Vol. 24, pp. 1703–1706, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

# CGS 8216, a Benzodiazepine Antagonist, Reduces Food Intake in Food-Deprived Rats

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BERNARD, P. S., G. PASTOR AND J. M. LIEBMAN. *CGS 8216, a benzodiazepine antagonist, reduces food intake in food-deprived rats.* PHARMACOL BIOCHEM BEHAV **24**(6) 1703–1706, 1986.—CGS 8216, a benzodiazepine receptor antagonist with weak inverse agonist properties, reduced food intake in food-deprived rats when administered orally or intraperitoneally at doses that antagonize diazepam. This effect was sustained when CGS 8216 was administered daily for five days, indicating no rapid tolerance to the anorectic effect. Ro 15-1788 did not reduce feeding when administered orally, and was active only at high intraperitoneal doses (54 and 100 mg/kg). CGS 9896, a close analog of CGS 8216 but a benzodiazepine partial agonist with anxiolytic properties, did not reduce food intake at doses as high as 100 mg/kg IP or PO. These results support prior suggestions that benzodiazepine receptors may modulate feeding behavior, and suggest that CGS 8216 may have appetite suppressant properties.

Feeding Benzodiazepines CGS 8216 CGS 9896 Ro 15-1788

BENZODIAZEPINE drugs reliably elevate food consumption under a wide variety of experimental conditions [7,9]. These findings have led to the hypothesis that benzodiazepine and/or gamma-aminobutyric acid receptors are involved in the regulation of food intake [9,16]. A corollary of this hypothesis would hold that drugs which antagonize benzodiazepine receptors, or which have effects opposite to those of benzodiazepine agonists, should reduce food intake. One such drug is CGS 8216, a pyrazoloquinoline that has benzodiazepine antagonist properties [4] as well as possible inverse agonist activity at benzodiazepine receptors [12]. The present experiments assessed the effects of CGS 8216 on food intake on food-deprived rats that were pretreated with drug prior to a daily one-hour feeding session.

For comparison, the effects of Ro 15-1788, a benzodiazepine antagonist that appears to lack inverse agonist properties [14], were assessed. In order to confirm the pharmacological specificity of the effects of CGS 8216, a structurally related compound, CGS 9896, was also tested. Unlike CGS 8216, CGS 9896 has partial benzodiazepine agonist properties which, in various behavioral assays, are expressed as mixed agonist/antagonist activity [1,2].

## METHOD

Male Wistar CRW (Charles River) rats (125–140 g) were individually housed, with food and water available ad lib, for a one-week accommodation period. At the start of experimentation, food was removed from the home cage but water continued to be available ad lib. Animals were then permitted to consume food only during a 1 hr daily feeding session. During feeding sessions, pre-weighed portions of laboratory rat food pellets were presented to each rat in a glass petri dish, and the amount of food not consumed at the end of the session was recorded. All spilled food was collected and added to the food remaining in the dish. Food consumption was measured as the amount of food presented minus non-consumed food.

Acute administration of drugs was performed after 4 days of this regimen. Prior to the fifth daily feeding session, rats were administered test drug as indicated below. Each drug experiment included a vehicle control for comparison. Each treatment group contained 10 animals. Food consumption after drug treatment was compared with that during the last previous feeding session. The mean percentage change in food consumption (±standard error) was calculated for each treatment group. Analysis of variance was performed on scores for percent change in food consumption, with comparisons of the effects of each dose to those of the corresponding vehicle control by Tukey's multiple comparison procedure [5].

In studies involving repeated drug treatment, the procedure for measuring food consumption was similar to that for acute treatment studies. Rats were allowed to consume food only during the 1 hr daily feeding session during two consecutive weeks, except for the intervening weekend when they were given a restricted amount of food (15 g of laboratory pellets) in their home cage each day. During the second week of this regimen, CGS 8216 (10, 30 or 100 mg/kg IP) or vehicle was administered daily 30 min prior to the feeding session for five consecutive days. The vehicle control group provided a basis for assessment of drug effects. During these experiments, animal body weights were recorded daily. Analysis of variance for repeated measures was performed separately on food consumption and body weight. Comparisons of mean values on each day were made using Tukey's HSD multiple comparison procedure [5].

Because of poor solubility, test drugs were prepared in a

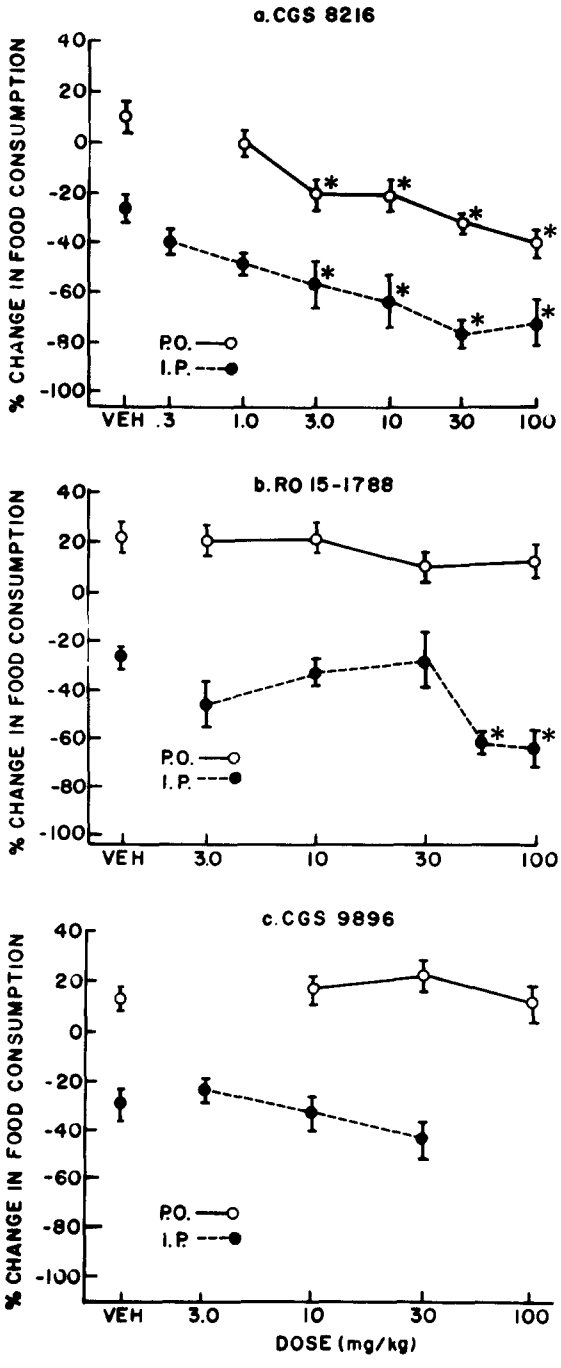


FIG. 1. Effects of acute administration of CGS 8216, Ro 15-1788 and CGS 9896 on food consumption in food-deprived rats. *Significantly different from vehicle control by Tukey's multiple comparison procedure, $p<0.05$.

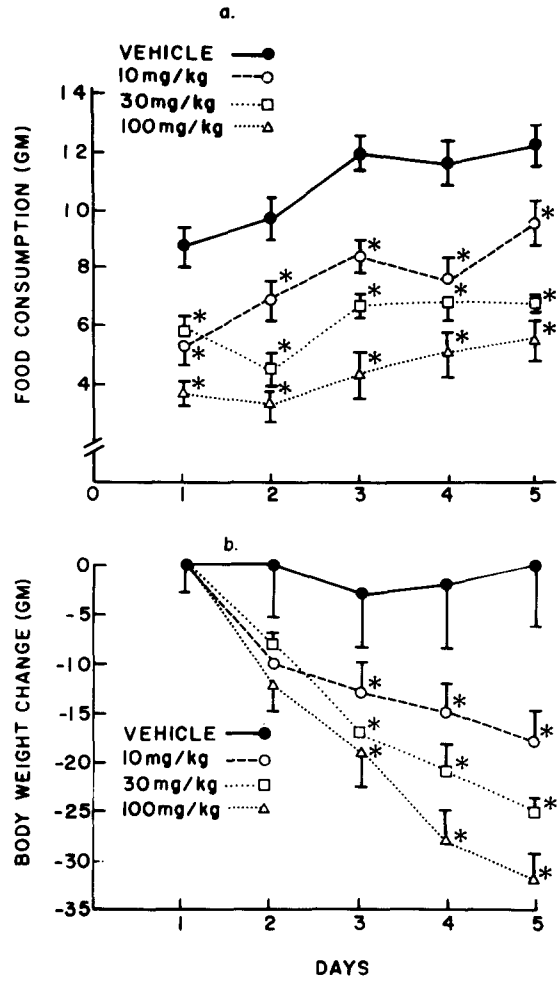


FIG. 2. Effects of repeated administration of CGS 8216 (IP) on food intake and body weight in rats. *Significantly different from vehicle control by Tukey's multiple comparison procedure, $p<0.05$.

3% (w/v) colloidal cornstarch suspension containing 5% (w/v) PEG-400 and 0.34% (w/v) Tween 80, and were administered either by oral intubation in a volume of 10 ml/kg body weight or intraperitoneally in 1.0 ml/kg body weight. Because of the short duration of action of Ro 15-1788 [15], the feeding session began immediately after administration of this drug. Feeding sessions were initiated 1 hr after oral intubation, or 30 min following intraperitoneal administration, of CGS 8216 and CGS 9896.

## RESULTS

Oral administration of the vehicle did not cause an appreciable change in food consumption by comparison with that during the last preceding feeding session (Fig. 1). Control groups consumed between 7 and 11 g of food during the 1-hr feeding periods. CGS 8216, after oral administration, caused a significant reduction in food consumption at 3.0 mg/kg (Fig 1a). Higher doses produced modestly larger reductions in feeding, but the total reduction in food consumption did not exceed approximately 40% at a dose of 100

mg/kg CGS 8216. Ro 15-1788 and CGS 9896 were ineffective at doses up to 100 mg/kg PO (Fig. 1b and 1c).

When the cornstarch vehicle was administered intraperitoneally, a small but consistent reduction was seen in food consumption (Fig. 1). At 3.0 mg/kg IP, CGS 8216 caused a further decrease in food intake, which was significant by comparison with the corresponding vehicle (Fig. 1a). Higher doses of CGS 8216 produced slightly greater reductions in food intake. No effect of Ro 15-1788 was noted at doses lower than 54 mg/kg IP (Fig. 1b). CGS 9896 did not reduce feeding significantly by the intraperitoneal route (Fig. 1c).

Repeated administration of CGS 8216 for 5 days at doses of 10, 30 or 100 mg/kg IP produced moderate, dose-related reductions in food consumption, which were sustained during the treatment period (Fig. 2a). Statistically significant reductions in body weight accompanied the changes in food intake (Fig. 2b).

## DISCUSSION

The present experiments demonstrate that CGS 8216 reduces food intake. These effects were apparent at low doses by both intraperitoneal and oral routes of administration. The minimum effective dose, approximately 1.0 to 3.0 mg/kg, was only slightly greater than the approximate $ED_{50}$ value for CGS 8216 to antagonize the anticonvulsant and muscle relaxant effects of diazepam [4]. The diazepam antagonist potency of CGS 8216 is comparable by oral and intraperitoneal routes (unpublished observations). The inhibition of feeding behavior by CGS 8216, therefore, occurred within a pharmacologically relevant dose range. Nevertheless, feeding was not completely suppressed by high doses of CGS 8216, up to and including a 100 mg/kg dose. By comparison, fenfluramine, a serotonin releaser which lacks benzodiazepine antagonist properties, is reported to reduce feeding in a dose-related fashion [3]. We have confirmed these findings under the present experimental conditions, with an 88% blockade of feeding behavior occuring at 10 mg/kg fenfluramine (unpublished observations by P. S. B.).

The effects of CGS 8216 were sustained for at least five days of daily intraperitoneal treatment, indicating that tolerance does not develop during this limited treatment period. Corresponing reductions in body weight were noted, as compared with the vehicle group. The effects of longer treatment periods remain to be evaluated.

When administered intraperitoneally, the cornstarch vehicle alone produced a small but consistent reduction in food intake. Oral administration of this vehicle did not reduce food intake despite the minimal caloric value of cornstarch. The effect of intraperitoneal administration of vehicle may be due to nonspecific peritoneal irritation or malaise. It is important to note that intraperitoneally administered CGS 8216 reduced feeding significantly even when compared with the effect of intraperitoneally administered vehicle alone.

CGS 9896 did not reduce food intake in the present paradigm. Although CGS 9896 has a very similar chemical structure to CGS 8216 and, like CGS 8216, binds avidly to brain benzodiazepine receptor binding sites, it appears to be a partial agonist with mixed agonist/antagonist properties, and not an inverse agonist [2]. These results, therefore, indicate that the reduction in feeding induced by CGS 8216 is related to its pharmacological mechanism of action rather than to some unsuspected aspect of the pyrazoloquinoline structure. The present experimental procedure does not permit assessment of drug-induced increases in feeding, because the food-deprived rats are presumably eating at maximal rates during exposure to test drugs. It is of interest that CGS 9896 either produces no effect [10] or only small, non-dose-related increases in feeding [18] when another procedure is used that reveals a robust facilitation of feeding by chlordiazepoxide.

The benzodiazepine antagonist, Ro 15-1788, also did not suppress feeding when administered orally, even when the experimental procedure was modified to allow for its short duration of action. When administered IP, Ro 15-1788 reduced feeding only at high doses (54 and 100 mg/kg). This finding has questionable pharmacological relevance since the effects of diazepam are completely antagonized by doses of Ro 15-1788 as low as 10 mg/kg IP or 30 mg/kg PO [4]. Since Ro 15-1788 is considered a benzodiazepine antagonist with slight agonist activity at higher doses, while CGS 8216 is considered a weak inverse benzodiazepine agonist [6], the inverse agonist activity appears crucial for the effects demonstrated.

The present results are consistent with the hypothesized involvement of benzodiazepine receptors in the regulation of feeding behavior [7, 9, 16]. Recently, Cooper and co-workers have shown that CGS 8216 and two other inverse benzodiazepine agonists reduce feeding behavior in a different procedure where non-deprived rats are presented with highly preferred foods during food consumption testing [8]. In agreement with the present results, these authors have reported that Ro 15-1788 has no effect by itself in this procedure despite its ability to antagonize diazepam-induced increases in feeding. One anomaly noted by these authors was that midazolam did not reverse the effects of CGS 8216 although the increase in feeding induced by midazolam was blocked by CGS 8216. A possible correlate of these findings is that the concentration of benzodiazepines required to displace $^3$H-CGS 8216 from binding sites is approximately four to six times higher than that required to displace $^3$H-diazepam or $^3$H-flunitrazepam, whereas CGS 8216 is equipotent in displacing all three ligands [11]. CGS 8216 may therefore have a noncompetitive interaction with benzodiazepine receptors or may interact with an additional receptor site not accessible to benzodiazepines.

The behavioral specificity of the effects of CGS 8216 remain to be fully elaborated. Although other operant behaviors such as intracranial self-stimulation are attenuated by CGS 8216 [17], these effects are selective, limited in magnitude [13], and do not extend to water intake [10]. It therefore seems reasonable to propose that CGS 8216 may have appetite suppressant properties and may represent a novel pharmacological approach to the treatment of obesity.

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