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BRIEF COMMUNICATION

A Novel Bombesin Receptor Antagonist Selectively Blocks the Satiety Action of Peripherally Administered Bombesin

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KIRKHAM, T. C., C. A. WALSH, J. GIBBS, G. P. SMITH, J. LEBAN AND J. McDERMED. *A novel bombesin receptor antagonist selectively blocks the satiety action of peripherally administered bombesin.* PHARMACOL BIOCHEM BEHAV **48**(3) 809–811, 1994. – To investigate the effect of a new, specific antagonist for bombesin receptors on the satiating action of exogenous bombesin, adult male rats were adapted to a nondeprivation test regimen with daily access to a palatable liquid food. In a prefeeding paradigm, rats received intraperitoneal injections of the bombesin receptor antagonist, BW2258U89 (6.25, 25, 50, or 100 $\mu g\ kg^{-1}$) or vehicle 20 min before, and then a second injection of either bombesin (4 $\mu g\ kg^{-1}$), gastrin-releasing peptide$_{18-27}$ (GRP_{18-27}; 16 $\mu g\ kg^{-1}$), the C-terminal octapeptide of cholecystokinin (CCK-8; 4 $\mu g\ kg^{-1}$), or vehicle 5 min before a 2-h feeding test. BW2258U89 pretreatment antagonized the satiating actions of bombesin and GRP18-27 in a very potent, dose-related manner, but did not antagonize the satiating action of CCK-8. These differential results with BW2258U89 are consistent with prior results showing the potency of this antagonist for bombesin receptor-mediated effects in visceral systems; in addition, they demonstrate the selectivity of the compound for the satiating actions of peripherally administered bombesin and bombesin-like peptides.

BW2258U89 Cholecystokinin Feeding behavior Food intake Gastrin-releasing peptide Satiation

AN analog of gastrin releasing peptide, [(des-NH_2)Phe^{19}, D-Ala^{24}, D-Pro$^{26}\Psi$ (CH_2NH)Phe^{27}]-GRP_{19-27} (BW2258U89), is a new bombesin-like peptide receptor antagonist. Singh et al. (10) reported that BW2258U89 is a highly potent antagonist at bombesin receptors in vitro and in vivo. The potency and specificity of this antagonist for the satiating action of peripherally administered bombesin-like peptides on food intake (2,3,5,8) have not been demonstrated. We report here that BW2258U89 inhibits this satiety action in a dose-dependent and selective manner. A preliminary report of aspects of this work has appeared (4).

METHOD

Ten male Sprague–Dawley rats, weighing 250–350 g, were housed singly and maintained on a 12 L : 12 D cycle (lights on at 0800 h). Prior to testing, rats were adapted to a nondeprivation test schedule. Using a paradigm adapted from Merali et al. (6), rats were given a 30-min prefeed (access to a palatable, high carbohydrate liquid test diet – 40% v/v L10007, Research Diets, Inc.) at 1100. At 1140 rats were injected intraperitoneally (IP) with either physiological saline as the vehicle control or BW2258U89 (100 $\mu g\ kg^{-1}$). A second injection of either saline vehicle or bombesin (4 $\mu g\ kg^{-1}$, IP) was administered at 1155. Rats were given the test diet at 1200 h. Intake was measured at 15, 30, 60, 90, and 120 min. On completion of this test, the same animals were retested to examine the ability of pretreatment with 6.25, 25, or 50 $\mu g\ kg^{-1}$ BW2258U89 to block the satiating effect of 4 $\mu g\ kg^{-1}$ bombesin. In addition, the selectivity of BW2258U89 toward specific bombesin receptors was examined by administering the antagonist in combi-

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nation with the C-terminal decapeptide of gastrin-releasing peptide (GRP18-27; Bachem, Torrance, CA), a mammalian bombesin-like peptide, or the synthetic C-terminal octapeptide of cholecystokinin (CCK-8; the generous gift of Bristol Meyers-Squibb, Princeton, NJ). Using the same design described above, rats were retested after administration of either GRP18-27 (16 $\mu g\ kg^{-1}$, IP) CCK-8 (4 $\mu g\ kg^{-1}$, IP), each alone and following pretreatment with an IP dose of 100 $\mu g\ kg^{-1}$ BW2258U89. The sequence of injection and intake measurements was as described above. The vehicle for each peptide solution was 0.15 M saline.

All rats received each treatment combination and acted as their own controls. Successive treatments with bombesin, CCK-8, GRP18-27, or BW2258U89 were separated by at least 48 h, with vehicle administered on intervening days. Because preliminary analysis revealed no reliable differences among successive vehicle treatments, data from those days were averaged for inclusion in the main statistical analyses. Because the major portion of food intake occurred in the first 30 min, intakes from this interval were analyzed using repeated-measures analysis of variance, with treatment and time as factors. Significance of differences between specific treatment means were assessed using Newman-Keuls test for multiple comparisons.

RESULTS

Peripheral administration of BW2258U89 produced a potent, dose-related reversal of the satiety action of peripherally administered bombesin. The figure displays the results during the first 30 min of the test, the period during which the first meal, and the majority of food intake, occurred. Note that the 4 $\mu g\ kg^{-1}$ dose of bombesin produced a reduction in intake of approximately 60%. Concomitant administration of a dose of BW2258U89 as low as 6.25 $\mu g\ kg^{-1}$ produced a significant attenuation of this satiety action. Increasing doses of BW2258U89 produced increasingly effective attenuations, and the largest dose, 100 $\mu g\ kg^{-1}$, produced a complete blockade. Given alone, this maximal dose of BW2258U89 had no effect on food intake.

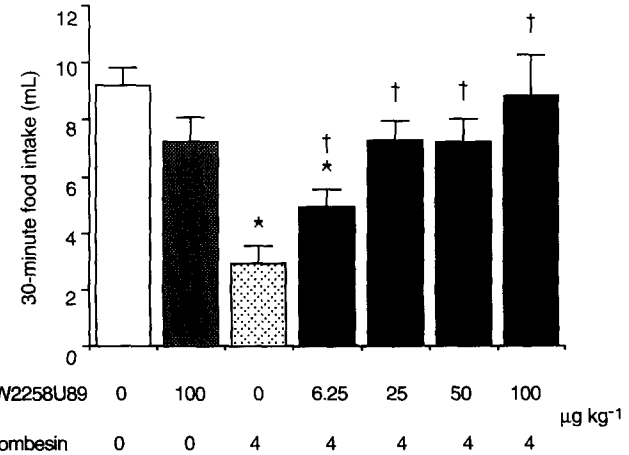

FIG. 1. Dose-related antagonism by BW2258U89 of bombesin-induced inhibition of liquid food intake. All values are mean ml (± SEM) 30-min liquid food intakes of 10 rats. Asterisks indicate that a treatment mean is significantly less than the vehicle/vehicle control ($p < 0.01$). Daggers indicate a significant reversal of bombesin effect by BW2258U89 ($p < 0.05$).

The antagonism provided by BW2258U89 was selective for bombesin-like peptides. The table compares the abilities of a dose of 100 $\mu g\ kg^{-1}$ of BW2258U89 to antagonize the inhibitory actions of three intraperitoneally administered peptides (bombesin, GRP18-27, and CCK-8). The antagonist reversed the inhibitory actions of GRP18-27 and bombesin, but had no effect on the inhibitory action of CCK-8.

DISCUSSION

The data reported here demonstrate that peripheral administration of the novel bombesin receptor antagonist BW2258U89 produces a dose-related antagonism of the satiating action of peripherally administered bombesin and of the mammalian bombesin-like peptide GRP18-27. In keeping with its demonstrated in vitro and in vivo actions in visceral systems (10), the antagonism of bombesin-induced satiety by BW2258U89 was extremely potent (Fig. 1).

The selectivity of antagonism provided by BW2258U89 for bombesin receptor-mediated satiety is demonstrated clearly by its failure to alter the satiating effect of peripheral administration of another gut/brain peptide, CCK (Table 1). BW2258U89 is an analog of GRP. In in vitro systems, it displays a 200-fold greater affinity for the GRP subtype of the bombesin-like receptor than for the neuromedin-B subtype (T. Moody, personal communication). Thus, the present results offer evidence that the GRP receptor mediates the satiating action of peripheral, exogenous bombesin, confirming and extending reports employing other bombesin receptor antagonists.

The largest dose of BW2258U89 employed here, 100 $\mu g\ kg^{-1}$, failed to increase food intake when administered alone (Fig. 1), supporting the view that BW2258U89 blocked bombesin- and GRP-induced satiety by blocking GRP receptors and not by increasing food intake through other, unidentified mechanisms. On the other hand, this result offers no support for a role for endogenous GRP in the physiological regulation of meal size. Using peripheral administration of another selective GRP receptor antagonist (D-Phe^6-bombesin$_{6-13}$ methyl ester) Reidelberger et al. (9) have reported, in preliminary form, increased food intake in rats. The reason for this difference, whether the use of a different antagonist or the fact that different conditions prevailed between the two experiments, is unclear at this point.

TABLE 1

COMPARISON OF ANTAGONISM BY BW22258U89 OF THE INHIBITION OF FOOD INTAKE BY THREE PEPTIDES

Peptide	Vehicle/Vehicle	Vehicle/Peptide	BW2258U89 Peptide
Bombesin ($4\ \mu g\ kg^{-1}$)	9.3 ± 0.6	3.0 ± 0.6*	8.8 ± 1.5
GRP_{18-27} ($16\ \mu g\ kg^{-1}$)	10.3 ± 1.5	4.4 ± 1.2*	8.3 ± 1.1
CCK-8 ($4\ \mu g\ kg^{-1}$)	8.8 ± 0.8	2.2 ± 0.4*	3.5 ± 1.1*

Values are mean ml (± SEM) of liquid food consumed in 30 min by 10 rats.

Intake following administration of BW2258U89/vehicle was 7.2 ± 0.9ml, a value not significantly different from control levels.

*$p < 0.01$, treatment significantly different from control (vehicle/vehicle) and from BW2258U89/vehicle.

Because BW2258U89 is an analog of GRP, and because neither bombesin nor GRP is known to cross the blood–brain barrier, it is probable that the site of antagonist action in the present report is peripheral, not central. It should be noted that other workers have demonstrated that the intracerebroventricular administration of bombesin receptor antagonists can block the satiety action of peripherally administered bombesin (7) and that intracerebroventricular antagonists at low doses can increase food intake when administered alone (1,6). How these clearly central actions relate to the likely peripheral actions described in the present report remains to be determined.

In summary, BW2258U89, a bombesin receptor antagonist with high affinity for the GRP receptor subtype, produced a very potent, dose-related, and selective blockade of the satiety action of exogenous, peripherally administered bombesin and GRP. BW2258U89 should provide an effective tool for examining the mechanism of action of bombesin-like peptides in feeding behavior, and for determining whether endogenous peptides of the bombesin family play a physiological role in this function.

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