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Meal Pattern Analysis in Rats Reveals Partial Agonist Activity of the Bombesin Receptor Antagonist BW2258U89

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KIRKHAM, T. C., J. GIBBS, G. P. SMITH AND N. GEARY. *Meal pattern analysis in rats reveals partial agonist activity of the bombesin receptor antagonist BW2258U89.* PHARMACOL BIOCHEM BEHAV **52**(1) 101-106, 1995.—The spontaneous feeding behavior of adult male rats was monitored during continuous infusion of the bombesin receptor antagonist BW2258U89 into the coeliac artery (100 $\mu g\ kg^{-1}\ h^{-1}$) from an osmotic minipump. Nocturnal food intake was suppressed over 5 days of testing. Similar effects followed acute BW2258U89 treatment [100 $\mu g\ kg^{-1}$, intraperitoneally (IP)]. Moreover, IP BW2258U89 mimicked the acute behavioral effects of 2 $\mu g\ kg^{-1}$ IP bombesin by reducing meal size. Verifying that BW2258U89 can retain its potency throughout the entire period of chronic infusion, we demonstrated that the acute anorectic action of bombesin (4 $\mu g\ kg^{-1}$, IP) was blocked by pretreatment with a BW2258U89 solution (100 $\mu g\ ml^{-1}\ kg^{-1}$, IP) that was freshly prepared, or incubated for 1 or 6 days at body temperature. These data demonstrate that acute and chronic administration of BW2258U89, at a dose that abolishes the satiety effect of bombesin, significantly suppresses spontaneous feeding. Thus, BW2258U89 appears to attenuate food intake by acting as a partial agonist at peripheral receptors for bombesin-like peptides.

Feeding Food intake Satiation Satiety Eatometer Gastrin-releasing peptide BW2258U89 Eating macrostructure Osmotic minipump Coeliac artery

PEPTIDES related to the amphibian peptide bombesin occur in both brain and periphery and may be involved in the satiation of eating. Administration of bombesin or its mammalian analogues, such as gastrin-releasing peptide (GRP) and neuromedin B, have been shown reduce food intake in laboratory animals (5) and human subjects (16). Specific receptor antagonists can attenuate the anorectic actions of peripherally or centrally administered bombesin-like peptides (10,13,14). Moreover, blockade of central bombesin receptors can induce a significant elevation of food intake, providing important support for a central role of these peptides in satiety (3,14).

One of the most potent bombesin receptor antagonists currently available is the modified GRP15-27 peptide, BW2258U89 (7,19). We have recently demonstrated that BW2258U89, injected intraperitoneally (IP), abolishes the satiety action of peripherally administered bombesin (10). In these experiments, we examined whether chronic, peripheral administration of BW2258U89 would produce significant alterations in spontaneous food intake, as with central antagonist treatment. Because gastric, and possibly other upper abdominal, sites perfused by the coeliac artery are particularly sensitive to the actions of bombesin (9), we attempted to maximize the influence of BW2258U89 on eating by administering the antagonist directly into the coeliac circulation.

METHOD

Animals

Eighteen adult male Sprague-Dawley rats (weighing 430 ± 26 g) were maintained on a 12 L : 12 D cycle (lights off at 1400 h) with free access to food and water.

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Eatometer Apparatus

Rats were individually housed in Plexiglas chambers and fed ground food (no. 5001; Purina Rat Chow, Purina Foods, Ralston, NC) ad lib. Food was obtained from containers set on electronic balances, situated beneath a small side chamber. A computer monitored the weight of food every 30 s, thus enabling analysis of individual meal patterns for each rat (4). Daily maintenance was performed during the light phase between 1100 and 1300 h.

Meal Pattern Analysis

Food intake was analyzed in terms of cumulative intake, meal size, meal duration, and intermeal interval. The criteria for defining meal parameters were as previously described (4,8,11). Specifically, a meal was defined as any episode of feeding lasting longer than 30 s, during which at least 0.3 g of food was consumed, and which was separated from the previous meal by at least 5 min.

Statistical Analysis

Effects of chronic BW2258U89 on individual meal parameters (meal size, meal duration, and intermeal interval) were assessed using a mixed analysis of variance (ANOVA) design with treatment and time as factors, followed by Newman-Keuls test for multiple comparisons. Acute, paired treatment effects and hourly cumulative intake data were analyzed using Student's *t*-test. Data obtained following acute vehicle, bombesin, or BW2258U89 injections (Experiments 2 and 3) were analyzed by ANOVA for repeated measures.

Peptides

For chronic administration (Experiment 1), the antagonist BW2258U89 ([des-NH_2)Phe^{19},D-Ala^{24},D-Pro$^{26}\Psi$(CH_2NH)Phe^{27}]-GRP_{19-27}) was dissolved in sterile 0.15 M saline. This solution was administered by means of 200 μl (Alzet 2001, Alza Corp., Palo Alto, CA) osmotic minipumps (see below for surgical details). The concentration of the antagonist solution was such that, at their nominal delivery rate of 0.95 μl h^{-1}, the minipumps provided an effective dose of 100 μg kg^{-1} h^{-1}. This dose was chosen to match, on a chronic basis, an acute peripheral treatment of BW2258U89 previously demonstrated to block the satiety action of peripherally injected bombesin (10). Additional tests were performed following acute, intraperitoneal injections of either bombesin (Bachem, Torrance, CA) or BW2258U89. Again, doses were chosen on the basis of previous experiments. Each solution was prepared using 0.15 M saline, and administered intraperitoneally (IP) in a volume of 1 ml kg^{-1} body weight. BW2258U89 was generously donated by Burroughs Wellcome Co. (Research Triangle Park, NC).

EXPERIMENT 1

Method

Surgery. After habituation to housing conditions, six animals were implanted with chronic polyurethane coeliac artery catheters. Catheters were made of Micro-Renathane tubing (OD 0.8 mm, ID 0.36 mm; Braintree Scientific, Braintree, MA). Each catheter tip (stretched and narrowed to the required diameter by heating in sesame oil at 200°C) was passed through a 2 × 2-mm square of preclotted DeBakey gauze (Bard Cardiosurgery Div., Billerica, MA), and then inserted through a needle-puncture wound into the artery. Catheters were anchored in the desired position with pigmented, polypropylene 9-0 (Prolene; Ethicon, Inc., Somerville, MA) microsuture, inserted through the ventral artery wall and tubing. In addition, the surrounding gauze was sutured to the artery wall to ensure a permanent haemostatic closure of the vessel. The distal end of the catheter was then passed subdermally, sealed, and temporarily exteriorized at the nape of the neck.

Four days later, after recovery from surgery and restoration of presurgery food intake levels (12), rats were lightly anaesthetized with Metaphane (Pitman Moore, Inc., Mundelein, NJ) and implanted with the osmotic minipumps. The catheters were filled with antagonist solution and connected subdermally to the pump outlet. The entire minipump-catheter assembly was concealed beneath the interscapular skin.

To control for the effects of surgical procedures, a second group of rats (n = 6) received laparotomies under general anesthesia, followed by a second, brief anesthetic treatment according to the same schedule described for the experimental group.

On completion of all testing, catheterized animals were again anaesthetized and the patency of each preparation was verified. Each minipump was removed and bisected to determine that drug delivery was complete. Finally, the abdominal organs were exposed, a dilute methylene blue solution was infused into the catheter, and the area of perfusion was recorded and compared with observations made at the time of the original surgery.

Design. Following minipump implantation (or control anaesthesia) on day 0, rats were deprived of food until dark onset the following day (1400 h). This delay before refeeding was imposed to ensure that the minipumps were actively delivering antagonist into the coeliac artery when testing began, and that the nutritional status of animals in experimental and control groups were equivalent. Meal patterns were subsequently recorded for a minimum of 5 days (the total duration of infusions was dependent upon the absolute volume of BW2258U89 solution injected into each pump, but in each case exceeded 6 days from the time of implantation).

On completion of these tests, we examined the extent to which prolonged, chronic antagonist infusion would affect the behavioral actions of an acute, peripheral dose of bombesin. On postoperative day 6, all rats were weighed and injected IP with 2 μg kg^{-1} bombesin, 15 min before dark onset. Feeding patterns were monitored for 12 h. This dose of bombesin falls near the anorectic threshold in tests where eating is motivated by palatability or deprivation, and was adopted in this test to facilitate observation of antagonism by BW2258U89.

RESULTS AND DISCUSSION

Chronic infusion of BW2258U89 produced an unanticipated suppression of food intake. This is illustrated most clearly by the cumulative intake during the dark phase, when rats typically consumed the major portion of their daily food intake. The effect was characterized on each subsequent test day by control levels of intake immediately after dark onset, but attenuated feeding throughout subsequent hours of darkness. This pattern was evident within 24 h after the start of infusion (Fig. 1A), and similar effects occurred through day 5 (Fig. 1B). Neither group ate substantial amounts during the light phase of testing, and no obvious difference between daytime eating patterns was apparent during the course of infusion.

The daily intake of each group gradually increased over the course of the experiment, primarily because of an increase in the total number of meals eaten on each successive day [F(4,

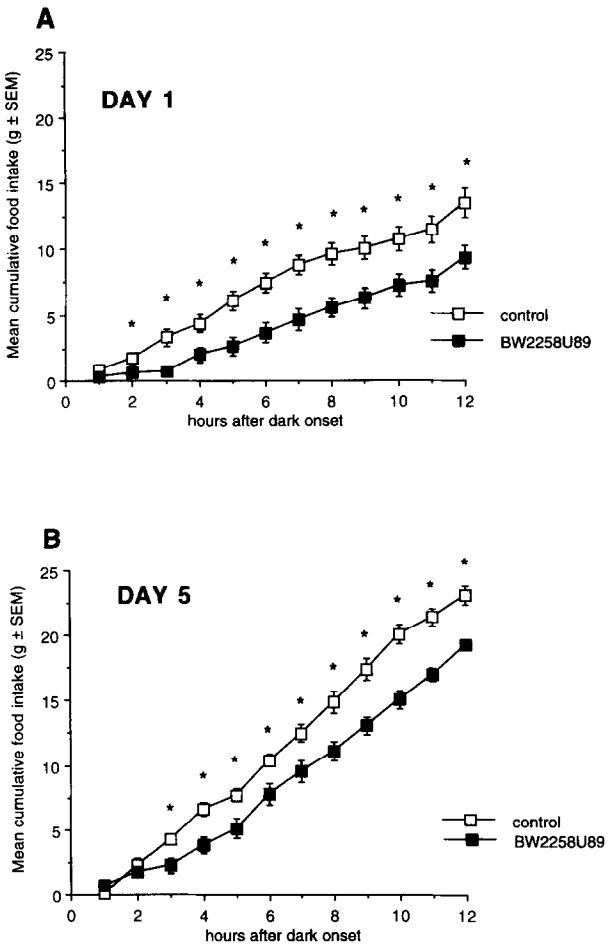

FIG. 1. Suppression of nocturnal food intake by BW2258U89 (100 $\mu g\ kg^{-1}h^{-1}$) infused chronically into the coeliac artery from an osmotic minipump, compared to sham-operated controls. All values are mean (±SEM) cumulative intake of six rats. *Significant difference between control and treatment mean at each interval ($p < 0.05$).

40) = 5.65, $p = 0.001$]. This effect was most marked in BW2258U89-treated rats (Table 1), in which meal frequency increased significantly from days 1–5 ($p = 0.018$). At the end of the study the totals for both groups were within the typical nocturnal range established for free-feeding rats (17,18). In contrast, meal size did not vary significantly (Table 1) between groups [$F(1, 10) = 1.57$, $p = 0.239$] or across time [$F(4, 40) = 2.50$, $p = 0.057$].

The gradual increase in daily intake may be related to the rate of recovery from the generalized postoperative effects of surgery. Prior to surgery, rats in both groups had similar patterns of feeding, with equivalent nighttime and 24-h intakes. General anaesthesia during catheterization or sham surgery produced a marked and persistent suppression of intake and body weight [as has been documented previously (12)]. However, before the second stage of surgery (day 0), both groups had similar daily intakes, which were not significantly different from those recorded before catheterization or laparotomy.

The reduced intake levels for both control and antagonist-treated animals on day 1 indicate that even brief inhalant anaesthesia may have a long-lasting influence on appetite. Nevertheless, the recovery of normal intake levels and meal frequency implies that any difference in the eating behavior between the groups at the end of the study may still be principally ascribed to the antagonist treatment. Moreover, the similarity of intake patterns during the first hours of darkness and the equivalent recovery of normal intake levels through day 5 are not supportive of the notion that the suppression observed in BW2258U89-treated rats resulted from some general toxicity.

In the second stage of this experiment, the influence of chronic BW2258U89 treatment on the effects of acute bombesin challenge were examined. When we compared controls and BW2258U89-treated rats, the most marked effect was on latency to the first meal after bombesin injection. Specifically, latency tended to be longer in controls (42 ± 8 min) than in animals receiving BW2258U89 infusion (21 ± 7 min, $p = 0.065$). Neither the size of the first meal of the dark nor any subsequent meal size or intermeal interval was affected by bombesin ($p > 0.2$), with similar values being obtained for both groups.

The tendency for bombesin to increase latency only in controls is consistent with antagonism by BW2258U89 of a satiety action of bombesin-like peptides. Because these rats were feeding ad lib, any augmentation of endogenous, meal-stimulated release of bombesin-like peptides might be expected to extend intermeal interval (reflected here by the latency measure; also see Experiment 3). Such an effect of peripherally administered bombesin on postmeal interval is well established (15,20). Consequently, the presence of BW2258U89 could oppose any bombesin-induced enhancement of intermeal satiety, producing a correspondingly shorter latency. The extended latency after bombesin, combined with the short half-life of the peptide by the IP route, could therefore underlie the lack of marked effects of bombesin on meal size or other parameters. Overall, the difference in latencies recorded in this test provide indirect evidence that BW2258U89 infusions were maintained throughout the experiment. Finally, it should be noted that postmortem examination of each minipump confirmed that all of their contents had been successfully delivered.

In summary, the principal effect of chronic BW2258U89 administration was to reduce spontaneous food intake, an opposite effect from that predicted on the premise that the antagonist acts at specific receptors within the periphery to block the actions of an endogenous satiety factor. On the basis of these results it is reasonable to assume that, although it retains its ability to block the acute effects of IP bombesin, BW2258U89 does not act entirely as an antagonist at receptors for bombesin-like peptides.

EXPERIMENT 2

The ability of chronic BW2258U89 infusion to suppress food intake suggests that the compound may have at least partial agonist activity. However, acute BW2258U89 injection, at a dose sufficient to block bombesin anorexia completely, failed to produce any significant effect on intake when administered alone (10). Thus, the possibility remains that BW2258U89 is a simple antagonist and that the feeding effects described earlier resulted from some adaptation induced by its prolonged administration at a high dose. For example, chronic antagonist blockade might result in upregulation of specific receptors for bombesin-like peptides, rendering the animals supersensitive to the actions of an endogenous ligand. Similar adaptations are well documented in other neural systems [e.g., (1,6,21)].

TABLE 1

DAILY AVERAGE MEAL SIZE AND TOTAL NUMBER OF MEALS OCCURRING DURING THE DARK PHASE ON EACH OF 5 SUCCESSIVE DAYS DURING CHRONIC INFUSION OF BW2258U89, OR FOLLOWING SHAM SURGERY

	DAY				
	1	2	3	4	5
Average meal size (g)					
Control	1.41 (0.09)	1.96 (0.37)	1.82 (0.19)	1.84 (0.18)	2.03 (0.15)
BW2258U89	1.37 (0.13)	1.57 (0.15)	1.71 (0.26)	1.68 (0.18)	1.57(0.10)
Total number of meals					
Control	8.17 (1.04)	7.83 (2.24)	11.17 (1.69)	10.33 (1.19)	10.67 (0.28)
BW2258U89	6.67 (1.04)	8.67 (2.24)	11.67 (1.69)	11.67 (1.19)	13.00 (0.28)*

All values are mean (±SEM). *Significant difference between day 1 and day 5 values ($p < 0.02$).

The following experiment addressed this issue by examining the acute effects of BW2258U89 on feeding macrostructure in drug naive-rats. The behavioral effects of BW2258U89 were also compared with those of the pure agonist bombesin. Assessing the immediate effects of BW2258U89 in this manner allowed for the observation of expression of agonist-like activity without the confounding influences of either surgery or prolonged exposure to BW2258U89. Furthermore, the sensitivity of the spontaneous meal pattern apparatus allowed us to detect subtle effects of BW2258U89 that might otherwise have been obscured by the abbreviated scope of a simple intake test.

Method

The acute effects of the antagonist on feeding behavior were assessed in a group of six male rats, using the apparatus described earlier. Animals were habituated to the eating chambers for several days until stable intake patterns were observed. On each test day, food was removed at 1000 h and withheld until dark onset (1400 h). This deprivation period was introduced to standardize the test across subjects, thus reducing the noise introduced by individual meal patterning. Subsequently, rats were weighed and injected IP with saline vehicle, 2 $\mu g\ kg^{-1}$ bombesin or 100 $\mu g\ kg^{-1}$ BW2258U89. Each injection was made 15 min before dark onset at 1400 h; intakes were monitored for the following 12 h. All animals received each treatment, administered in counterbalanced order, on consecutive days.

Results and Discussion

As with chronic administration, acute BW2258U89 treatment significantly suppressed food intake, although the time course of the effect was somewhat different. In line with its peptidergic nature and mode of administration, BW2258U89 had both a rapid onset and short duration of action: Marked suppression of eating was evident only during the first hour after administration.

The antagonist and bombesin each reduced intake to a similar extent (Fig. 2). Moreover, meal pattern analysis revealed that BW2258U89 and bombesin attenuated food intake through similar adjustments to meal parameters (Table 2). Both substances markedly reduced initial meal size [$F(2, 10) = 5.136$, $p = 0.029$], associated with a tendency for meal duration to be reduced [$F(2, 10) = 3.397$, $p = 0.075$]. In addition, although not approaching significance, both BW2258U89 and bombesin tended to increase the latency to this first meal. After the first hour of feeding, animals compensated for the lost intake so that total 2-h intakes were comparable after each of the three treatments.

By removing the potential confounding consequences of anaesthesia/surgery-induced malaise, or neural adaptation following chronic receptor blockade, these data strengthen the conclusion suggested by Experiment 1, that BW2258U89 has agonist properties.

EXPERIMENT 3

Although the alterations to feeding macrostructure in rats receiving chronic BW2258U89 infusion were consistent across the whole course of Experiment 1, we felt it necessary to demonstrate that the antagonist does indeed retain its potency throughout the course of chronic administration. Therefore, the following experiment was intended to simulate the combined effects of time and prolonged incubation at body temperature on antagonist solutions delivered over days from the osmotic minipumps. The stability of the BW2258U89 solution was examined by testing the antagonist's ability to block bombesin anorexia at various intervals after its preparation.

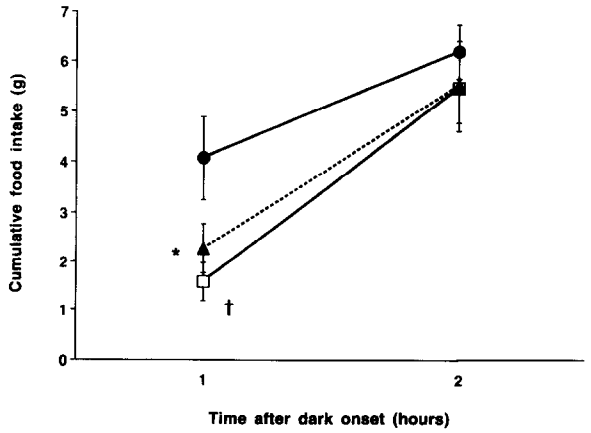

FIG. 2. Nocturnal cumulative intake after acute (IP) treatment with saline, 100 $\mu g\ kg^{-1}$ BW2258U89, or 2 $\mu g\ kg^{-1}$ bombesin. All values are mean (±SEM) of six rats. *Significant difference between saline and BW2258U89 treatments ($p < 0.05$). †Significant difference between saline and bombesin treatments ($p < 0.05$).

TABLE 2

EFFECTS OF IP BOMBESIN OR BW2258U89 ON ONSET LATENCY, SIZE, AND DURATION OF THE FIRST MEAL AFTER DARK

	Latency (min)	Meal Size (g)	Meal Duration (min)
Vehicle	12.2 (5.1)	4.07 (0.82)	16.7 (3.1)
2 μg kg^{-1} Bombesin	22.2 (10.8)	1.93 (0.44)*	9.3 (3.1)
100 μg kg^{-1} BW2258U89	26.0 (11.7)	1.97 (0.41)*	7.7 (2.3)

All treatments were administered 15 min before lights out. All values are mean (±SEM) of six rats. *Significant difference from vehicle value ($p < 0.01$).

Method

A solution of 100 μg ml^{-1} BW2258U89 solution was prepared and separated into three portions: one for immediate administration and the remainder for injection following 1 or 6 days' incubation in a water bath at 37.5°C. The potency of the antagonist solution at each point was determined by administering it before the injection of bombesin.

At 1300 h on each test day, 60 min before each intake test, food was removed from unoperated rats ($n = 6$). At 1340 h, rats were injected (IP) with saline or BW2258U89 solution (in a volume of 1 ml^{-1} kg^{-1} (i.e., a dose of 100 μg kg^{-1}), followed at 1355 h by a second injection of either saline or 4 μg ml^{-1} kg^{-1} bombesin. At 1400 h, rats were given 60 min access to a liquid diet (40% v/v L10007, Research Diets, Inc., New Brunswick, NJ). Combined treatments were scheduled so that all animals were tested with freshly prepared, 24 h- and 6-day incubated BW2258U89 solution, and that successive peptide treatments were preceded by a vehicle control day. As vehicle baselines did not vary significantly across the study, control day mean values were used in the statistical analysis of the peptide data.

In addition, saline-bombesin tests were performed both before and after the series of combined BW2258U89-bombesin treatments. The results of these two bombesin treatments were compared to assess whether any attenuation of bombesin's satiety action in combination with BW2258U89 involved direct antagonism, rather than desensitization to bombesin after its repeated administration (2).

Results and Discussion

Sole administration of bombesin produced marked (~40%) suppression of intake before and after completion of the interaction tests ($p < 0.01$). Thus, bombesin retained its full potency across the entire course of the study, and the following results may be considered in terms of the relative antagonist potency of BW2258U89, rather than being due to development of tolerance to bombesin. Figure 3 compares the effects of separate and combined treatment of antagonist and agonist on liquid diet intake.

At each pairing, BW2258U89 effectively abolished the ability of bombesin to suppress food intake, confirming our previous findings (10). Moreover, there was no apparent deterioration in antagonist potency, even after 6 days of incubation. Thus, these data suggest that BW2258U89 delivered chronically by osmotic minipump was effective throughout all stages of Experiment 1 and, by extension, further support the general conclusion from these studies that BW2258U89 possesses partial agonist efficacy.

GENERAL DISCUSSION

Endogenous bombesin-like peptides such as GRP and neuromedin B are believed to comprise an important component of preabsorptive satiety signals (5). The GRP analogue BW2258U89 has previously been characterized, using in vitro and in vivo assays, as one of the most potent bombesin receptor antagonists yet to be developed (19). We previously demonstrated that BW2258U89 can provide total protection against the intake-suppressing action of peripherally administered bombesin (10). Because of this potency of BW2258U89 to antagonize exogenous bombesin, in the present study we examined whether BW2258U89 would also block the actions of endogenous, peripherally released, bombesin-like peptides. We anticipated that if these peptides are indeed involved in satiation, BW2258U89 alone should replicate the hyperphagic actions of centrally injected bombesin-receptor antagonists reported elsewhere (3,14). We adopted the sensitive spontaneous

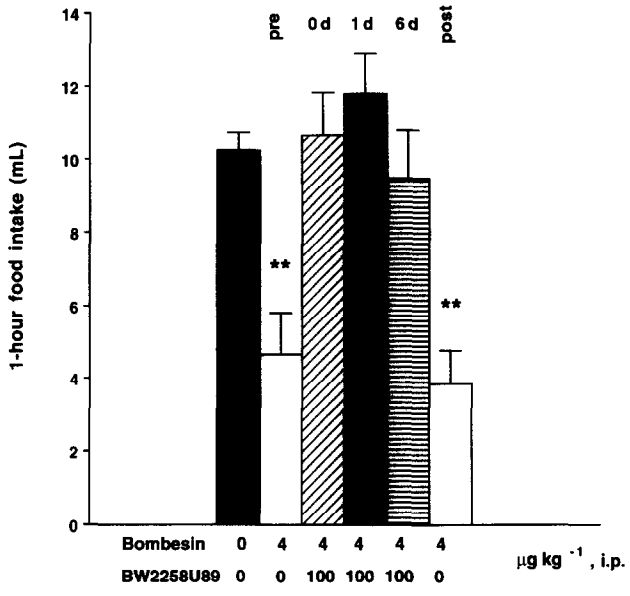

FIG. 3. BW2258U89 retains its antagonist potency, compared to a freshly prepared solution (0d), after incubation at body temperature for 1 or 6 days (1d and 6d). Antagonist data are contrasted with the effects of bombesin alone tested before (pre) and after (post) the combined BW2258U89-bombesin tests. All values are mean (±SEM) of six rats. *Significant difference between control and treatment means at each interval ($p < 0.01$).

feeding paradigm to maximize our capacity to detect subtle changes to feeding patterns not amenable to analysis using more routine techniques.

Contrary to our expectations, BW2258U89 actually produced a significant suppression of spontaneous feeding in catheterized rats receiving chronic infusion of BW2258U89. We also obtained an anorectic response in naive rats treated acutely with BW2258U89, indicating that the inhibitory effect of chronic BW2258U89 administration was a primary effect of the antagonist, rather than some secondary consequence of the persistent blockade of receptors for bombesin-like peptides. Moreover, meal pattern analysis revealed that the acute effects of BW2258U89 mimicked the behavioral effects of bombesin, such that similar reductions in consumption after each treatment were obtained through very similar adjustments to the separate meal parameters.

The similarities between acute and chronic responses to BW2258U89 suggest that BW2258U89 attenuates food intake by acting as a partial agonist at peripheral receptors for bombesin-like peptides. Partial agonist activity of BW2258U89 is also supported by the fact that despite their similar molecular weights, a dose of 100 $\mu g\ kg^{-1}$ BW2258U89 that abolished the anorectic action of bombesin (Experiment 3) was no more effective than a dose of 2 $\mu g\ kg^{-1}$ of bombesin at attenuating food intake.

Although pharmacodynamic data on BW2258U89 generally support a competitive antagonist profile, there is a least one precedent for a partial agonist action of the drug. For example, Singh et al. (19) reported that in dogs (but not rats), intravenous infusion of BW2258U89 elevated plasma gastrin levels. The effect was slight compared to the gastrin elevation produced by bombesin infusion, but was nonetheless significant.

Although an agonistic component to the action of BW2258U89 could reasonably account for the suppression of intake when administered alone, the potent antagonist properties of the drug might still be expected to exert significant blockade of the endogenous ligand. If that ligand was a component of a peripherally generated satiety signal, competition for receptor occupancy with BW2258U89 ought to result in a net increase in consumption. That BW2258U89 consistently suppresses the intake of free-feeding rats—in the absence of exogenous bombesin—suggests that a more complicated account is required to explain these phenomena. The nature of that complexity remains to be determined, and must take into account the probable involvement of, and interaction with, related peptides (such as neuromedin B) and the respective receptor populations at which they act. However, because BW2258U89 has a high affinity for gastrin-releasing peptide receptors, its ability to affect intake in either direction provides general support for the hypothesized role of bombesin-like peptides in satiety.

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