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Intravenous infusion of gastrin-releasing peptide-27 and bombesin in rats reveals differential effects on meal size and intermeal interval length

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ABSTRACT

We have previously shown that the intraperitoneal (i.p.) administration of gastrin-releasing peptide-27 (GRP-27) or bombesin (BN) (at 0.21, 0.41 and 1.03 nmol/kg) reduces meal size (MS) and prolongs the intermeal interval (IMI). Here, we hypothesized that the intravenous (i.v.) administration of the same doses of GRP-27 and BN will be as effective as the i.p. administration in evoking these feeding responses. To test this hypothesis, we administered GRP-27 and BN i.v. and measured first MS (10% sucrose), IMI, satiety ratio (SR, IMI/MS) and second MS in overnight food-deprived but not water-deprived male Sprague Dawley rats. We found that (1) only GRP-27 reduced the first MS, (2) BN prolonged the IMI, (3) GRP-27 and BN increased the SR and (4) only BN reduced the size of the second meal. Contrary to our hypothesis, the i.v. administration of GRP-27 and BN affected the MS and IMI differently than did the i.p. administration. In conclusion, this pharmacological study suggests that the MS and IMI are regulated at different sites.

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1. Introduction

Bombesin (BN) is a 14-amino-acid peptide that was isolated from the skin of the European frog *Bombina bombina*. Homologs of this peptide, which are secreted from the enteric neurons of mammals, include three forms of gastrin-releasing peptide (GRP) and a 10-amino-acid peptide referred to as neuromedin B (NMB). The three forms of GRP include GRP-10, the small form of GRP that exists in all species; GRP-27, the large form of GRP that exists in all species except rats; and GRP-29, the large form of GRP that exists only in rats [25].

Bombesin and GRP activate three G protein-coupled receptors to evoke their responses. These receptors are GRP-R or BB_1, NMB-R or BB_2 and an orphan Bn receptor subtype-3 (BRS-3) or BB_3. Gastrin-releasing peptides and BN bind the BB_1 receptor. However, BN has approximately thirteen times more affinity for the BB_1 receptor than does GRP (34 nM for BN vs. 440 nM for GRP). The activation of these receptors by BN and GRP evokes responses such as hyperthermia, bradycardia, inhibition of gastric emptying and inhibition of food intake [25].

The reduction of food intake by BN and GRP has been previously investigated. Intraperitoneal (i.p.) injections of BN and GRP reduce the meal size (MS) and prolong the intermeal interval (IMI) length [5,29,36]. Furthermore, Rushing et al. [24] have shown that the intravenous (i.v.) administration of GRP-27 (at 5 and 10 μg/kg) following the end of the first meal to freely feeding, non-disturbed rats prolongs the IMI.

Possible pathways for the previously identified GRP- and BN-induced feeding responses have been evaluated. For example, reduction of the MS and prolongation of the IMI by BN and GRP-29 require vagal, capsaicin-sensitive, and splanchnic nerves [15,28,31,37]. Such responses are accompanied by increased Fos-like immunoreactivity (Fos-LI, a marker for neuronal activation) in the enteric neurons of the stomach and the duodenum [35] and in food control areas in the hindbrain [14–16], including the area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV) [13,35].

Despite the previous data, two questions remain unanswered. First, what is the site(s) of action that regulates the MS and IMI length by GRP? Second, are these feeding responses regulated by the same site or different sites? To answer these questions, we hypothesized that if the MS and IMI length are regulated by the same site(s), then administering the peptides via i.v. will produce similar effects as did the i.p. administration, which we previously tested [36] using the same doses and experimental design. On the

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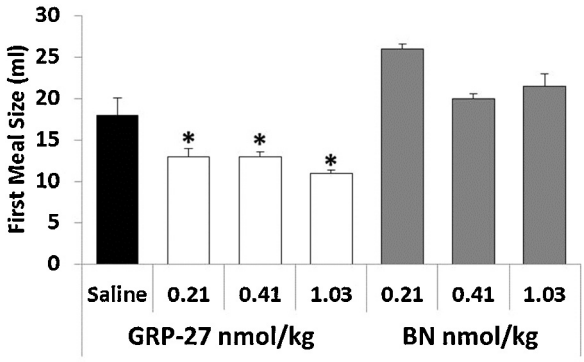

Fig. 1. Effect of the intravenous infusion of gastrin-releasing peptide-27 and bombesin on the first meal size. Gastrin-releasing peptide-27 (GRP-27) and bombesin (BN) (0, 0.21, 0.41 and 1.03 nmol/kg) were infused in the femoral vein of overnight food-deprived Sprague Dawley rats ($n = 8$), and the first meal size (MS, 10% sucrose) was determined. Only GRP-27 (all doses) reduced the MS relative to the effect of the saline vehicle (* denotes the significance, $p < 0.05$). Black bar: saline, empty bars: GRP-27, gray bars: BN.

other hand, if the MS and IMI length are regulated by different sites, then administering GRP-27 and BN via i.v. will affect the MS and IMI length differently than did the i.p. administration.

To test this hypothesis, we equipped overnight food- but not water-deprived rats with i.v. catheters in the femoral vein (FV) and infused them with various doses of GRP and, for comparative purposes, BN. Immediately following the infusion, we measured their food intake (10% sucrose) and rated their behavior every minute to determine the size of the first two meals, the time between them (the IMI), and the satiety ratio (SR; IMI (min)/MS (ml) – defined as the amount of food that is consumed per a given unit of time). The current study suggests that the regulation of the MS and IMI length by GRP-27 and BN occurs at different sites, which we propose to be gastrointestinal sites (Figs. 1–3).

2. Materials and methods

2.1. Experimental procedures

The Tuskegee University Institutional Animal Care and Use Committee approved the protocols for all of the experiments (R1108-14-1). Adult male Sprague Dawley rats ($n = 8$, 350–400 g; Harlan, IN, USA) were housed individually in special plastic cages

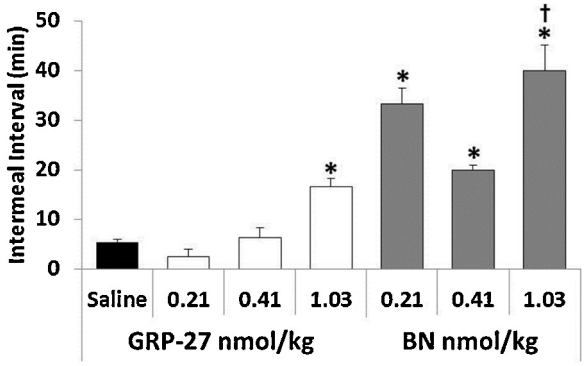

Fig. 2. Effect of the intravenous infusion of gastrin-releasing peptide-27 and bombesin on the length of the intermeal interval. Gastrin-releasing peptide-27 (GRP-27) and bombesin (BN) (0, 0.21, 0.41 and 1.03 nmol/kg) were infused in the femoral vein of overnight food-deprived Sprague Dawley rats ($n = 8$), and the time between the first and the second meals (intermeal interval, IMI) was determined. GRP-27 (1.03 nmol/kg) and BN (all doses) prolonged the IMI relative to the effect of the saline vehicle (* denotes the significance, $p < 0.05$). Black bar: saline, empty bars: GRP-27, gray bars: BN.

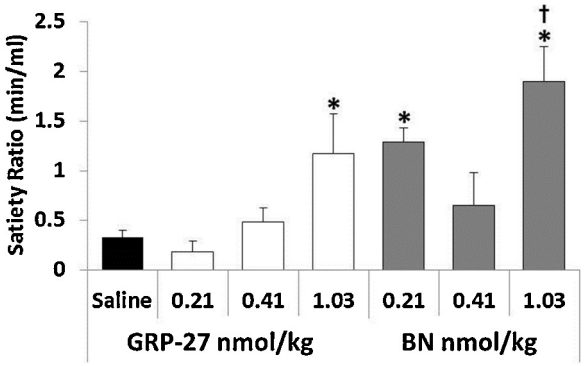

Fig. 3. Effect of the intravenous infusion of gastrin-releasing peptide-27 and bombesin on the satiety ratio. Gastrin-releasing peptide-27 (GRP-27) and bombesin (BN) (0, 0.21, 0.41 and 1.03 nmol/kg) were infused in the femoral vein of overnight food-deprived Sprague Dawley rats ($n = 8$), and the satiety ratio (SR; the time between two meals (the intermeal interval in minutes) divided by the first meal size in ml was determined. GRP-27 (1.03 nmol/kg) and BN (0.21 and 1.03 nmol/kg) increased the SR relative to the effect of the saline vehicle (* denotes the significance, $p < 0.05$), and BN at 1.03 nmol/kg increased this ratio more than the same dose of GRP-27 († denotes the significance, $p < 0.05$). Black bar: saline, empty bars: GRP-27, gray bars: BN.

to allow for the visualization of the animals from all sides for complete behavioral analysis during the food intake experiment. The rats were housed in a controlled environment with a 12 h light/12 h dark cycle at a temperature of 21 °C and given free access to rodent chow pellets and water. To adapt the animals to the experimental protocol, each rat was handled daily for at least 10 min and given a 10% sucrose solution in addition to rat chow and water, and the catheters were flushed with heparinized saline.

2.2. Surgical procedure

Eight animals underwent femoral vein (FV) catheterization using a surgical microscope (*Carl Zeiss OPMI 160*, *12.5x/18B*, *1x250*), general anesthesia (1 mg/kg body weight, intramuscularly prepared in our laboratory by mixing 5.0 ml Ketaset (100 mg/ml), 2.5 ml Rompun® (xylazine 20 mg/ml), 1.0 ml acepromazine maleate® (10 mg/ml) and 1.5 ml saline) and an incision in the medial aspect of the thigh. Following the absence of the pedal reflex, which denotes the surgical stage of anesthesia and the animal readiness for surgery, the medial surface of the thigh was clipped and surgically prepared alternatively with Betadine solution and alcohol swabs three times for three minutes each time. A 3-cm incision was made in the medial aspect of the right thigh to expose the FV. Following the complete freeing of the vein from the surrounding tissue/fascia, a microvascular clamp (*Microsurgery Instruments*, *Inc.* MC6 double clamp 0.9 cm) was applied to the vein (*Acland's Practice Manual for Microvascular Surgery by Robert D. Acland*, *2008*). The FV was then punctured just below the base with a sterile 30-gauge needle, and the catheter (Micro-Renathane *Braintree Scientific* MRE-025 .025 O.D.X.012 I.D.) was threaded into the vein. The catheter was then fixed into place using a non-absorbable suture material (stay suture, silk, size 6-0, *Ethicon*) and sealed at the point of entry by applying cyanoacrylate glue (super glue) to the catheter and vein, and the clamp and the stay suture were removed to allow blood flow to the vein. The catheter was then threaded out subcutaneously to the back of the head between the shoulder blades (scapula), where it was secured with sutures and super glue. All of the catheters were flushed twice daily with heparinized saline (0.5 ml). The muscles of the thigh region were closed using a polydioxanone II (5-0) absorbable suture material, and the skin was closed using surgical staples. Postoperative care included Metacam® (Meloxicam® (1.1 mg/kg)) subcutaneously for