Hormones and Behavior 85 (2016) 1–4

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

The BB_2 receptor antagonist BW2258U89 attenuates the feeding responses evoked by exogenous gastrin releasing peptide-29

Martha C. Washington, Thaer R. Mhalhal, Ayman I. Sayegh *

Gastroenterology Laboratory, Department of Biomedical Sciences, College of Veterinary Medicine, Tuskegee University, Tuskegee, AL 36088, USA

ARTICLE INFO

Article history:
Received 4 April 2016
Revised 29 June 2016
Accepted 30 June 2016
Available online 02 July 2016

Keywords:
GRP
Meal size
Intermeal interval
Satiety ratio
Celiac artery

ABSTRACT

This confirmatory work is aimed to test that the hypothesis that the gastrin releasing peptide (GRP) receptor – the BB_2 receptor – is necessary for reduction of meal size (MS) and prolongation of the intermeal interval (IMI) by the small and the large forms of GRP in the rat, GRP-10 and GRP-29, and to confirm the sites of action regulating such responses – the vascular bed of the celiac artery (CA, supplying stomach and upper duodenum). To pursue these aims we measured first MS and IMI length in response to GRP-10 and GRP-29 (0, 0.5 nmol/kg) infused in the CA ($n = 8$ rats) and the cranial mesenteric artery (CMA, supplying the small and part of the large intestine, $n = 8$ rats) in near spontaneously free feeding rats pretreated with the BB_2 receptor antagonist BW2258U89 (0.1 mg/kg) in the same arteries prior to the onset of the dark cycle. We found that GRP-29, but not GRP-10, infused by the CA reduced MS and prolonged the IMI by decreasing meal latency and meal duration and the BB_2 receptor antagonist BW2258U89 infused in the same artery attenuated these responses. These results suggest that the BB_2 receptor is necessary for reduction of MS and prolongation of the IMI by exogenous GRP-29, and the vascular bed of the CA, stomach and upper duodenum, contains sites of action regulating these feeding responses.

1. Introduction

Gastrin releasing peptides (GRP) are mammalian gut peptides secreted mainly by the nerves of the gastrointestinal tract, the enteric nervous system, and have strong sequence homology with the amphibian skin peptide bombesin (Bn). Based on the number of amino acids in these peptides there are three molecular forms of GRP: GRP-10, GRP-27 and GRP-29. In the rat GRP-10 and GRP-29 are the small and the large molecular forms of GRP respectively (Sayegh, 2013).

Gastrin releasing peptides evoke a number of physiological responses (Gonzalez et al., 2008) e.g. hormonal release, smooth muscle contraction and trophic action by activating three G protein coupled receptors, BB_1, BB_2 and BB_3, distributed both centrally and peripherally. Gastrin releasing peptide binds the BB_1 receptor less potently than the BB_2 receptor (148 and 0.19 nM, respectively). Therefore, when doing experiments with GRP one must remember that the difference in the binding affinity between the two receptors is 779 folds (Ramos-Alvarez et al., 2015). This makes the BB_2 receptor antagonist crucial for assigning actions of GRP to the BB_2 receptor. The BB_3 receptor, or the orphan receptor, binds GRP and Neuromedin B, another Bn-related peptide, but extremely weakly (>3000 nM) (Ramos-Alvarez et al., 2015).

The role of GRP in the short term control of food intake, reduction of individual meal size (MS) and prolongation of the intermeal interval (IMI or time between two consecutive meals), has also been evaluated. Others and we have shown that GRP-10 and GRP-29 reduce MS and prolong the IMI (Reeve et al., 2014; Stein and Woods, 1982; Washington et al., 2014b, 2011). In addition, reduction of food intake by GRP-10 was attenuated by the BB_2 receptor antagonist (Ladenheim et al., 1996). However, the role of this receptor in reduction of food intake by the large form of GRP or GRP-29 has not been examined prior to this work.

Furthermore, the site of action controlling MS and IMI length by exogenous GRP has also been examined (Washington et al., 2014a). We have shown that the vascular bed of the celiac artery (CA, supplying the stomach and the upper duodenum) contains sites of action controlling MS and IMI length by exogenous GRP-29 but not GRP-10. However, this result has not been confirmed by infusing the GRP, BB_2, receptor antagonist in the same artery, which we performed in the current study.

Based on our previous work (Washington et al., 2014a) the current study measured MS (normal rat chow), IMI length, latency to first meal, duration of first meal, total number of meals and total food intake during a 24 h period by GRP-10 and GRP-29 (0.5 nmol/kg, the highest effective dose) infused in the CA and the cranial mesenteric artery (CMA, supplying small and part of the large intestine) in near

* Corresponding author at: Gastroenterology Laboratory, Department of Biomedical Sciences, College of Veterinary Medicine, Tuskegee University, Tuskegee, AL, 36088, United States.
E-mail address: sayeghai@mytu.tuskegee.edu (A.I. Sayegh).

http://dx.doi.org/10.1016/j.yhbeh.2016.06.012
0018-506X/

spontaneously free feeding rats pretreated with the specific BB_2 receptor antagonist BW2258U89 (0.1 mg/kg (Kirkham et al., 1994)) in the same arteries prior to the onset of the dark cycle. The BW2258U89 receptor antagonist is a specific GRP, BB_2, receptor antagonist which belongs to a class of bombesin receptor antagonists (modified GRP(15–27) peptides, with D-Pro26 and D-Ala24 moieties). The BW2258U89 ([de-NH2)Phe19,D-Ala24,D-Pro26 psi(CH2NH)Phe27]-GRP(19–27)) was most potent towards inhibiting insulin and gastrin release in dogs and rats respectively.

Our results show that the BB_2 receptor is necessary for reduction of MS, prolongation of the IMI, reduction of first meal latency and reduction of first meal duration by GRP-29. In addition, the site of action controlling MS and IMI length by GRP-29 is located in the vascular bed of the CA.

2. Materials and methods

The Tuskegee University Animal Care and Use Committee approved all animal protocols. Adult male Sprague Dawley rats weighing 400–450 g ($n = 16$, divided into CA, CMA groups, $n = 8$ each) were individually housed in the BioDAQ E2 system (Research Diets, New Brunswick, NJ) in a controlled environment (12 h dark/12 h light cycle — lights off at 1800 h, 21.5 °C), with water and pelleted rodent chow (Teklad, Madison, WI) available ad libitum.

2.1. Vascular catheterization, meal patterns and habituation:

One catheter was implanted in each rat, and the meal patterns (meal was consumption of ≥0.2 g, and intermeal interval [IMI] was no feeding activity for ≥15 min), were done as described in details previously (Sayegh et al., 2015; Washington et al., 2014a, 2015; Williams et al., 2016).

On Mondays, Wednesdays and Thursdays at 1750 h rats received a heparinized saline infusion. On Tuesdays, Thursdays and Saturdays, the rats received BW2258U89 (0.1 mg/kg) at 1750 h followed by GRP-10 or GRP-29 (0.5 nmol/kg) at 1800 h. Sundays were reserved for the maintenance, but the catheters were flushed with 0.3 ml of the heparinized saline solution twice a day including Saturdays and Mondays. Treatments were done in random order.

2.2. Statistical analysis

Meal size, IMI and SR were analyzed individually using two-way analyses of variance (route × treatment, with repeated measures on treatment), followed by Bonferroni-corrected *t*-tests for pairwise comparisons. Results were considered significant if $p < 0.05$.

3. Results

3.1. GRP-10

3.1.1. MS, IMI, SR, latency and duration

ANOVA for each response revealed no main effects of treatments, routes or treatments and routes interaction.

3.1.2. Total intake

ANOVA revealed a main effect of treatment ($F_{3, 42} = 4.99$, $p = 0.005$) $\eta^2 = 0.18$ and route ($F_{3, 42} = 4.76$, $p = 0.047$) $\eta^2 = 0.07$.

3.1.3. Number of meals

ANOVA revealed a main effect of treatment ($F_{3, 42} = 3.09$, $p = 0.037$) $\eta^2 = 0.14$.

3.2. GRP-29

3.2.1. MS

ANOVA revealed a main effect of treatment ($F_{3, 42} = 7.48$, $p < 0.001$) $\eta^2 = 0.27$. Follow-up tests revealed that V/GRP-29 infused via the CA reduced the first MS relative to V/S ($p = 0.002$) and A/GRP-29 attenuated this effect ($p = 0.001$) (Fig. 1, upper panel).

3.2.2. IMI

ANOVA revealed a main effect of treatment ($F_{3, 42} = 4.56$, $p = 0.05$) $\eta^2 = 0.09$. V/GRP-29 infused via the CA increased the IMI relative to V/S ($p = 0.01$) and A/GRP-29 infused via the CA attenuated this response ($p = 0.008$) (Fig. 1, middle panel).

3.2.3. SR

ANOVA revealed a main effect of treatment ($F_{3, 42} = 5.32$, $p = 0.003$) $\eta^2 = 0.21$ and follow-up tests revealed a significant increase of SR by V/GRP-29 infused via the CA relative to V/S ($p = 0.005$)

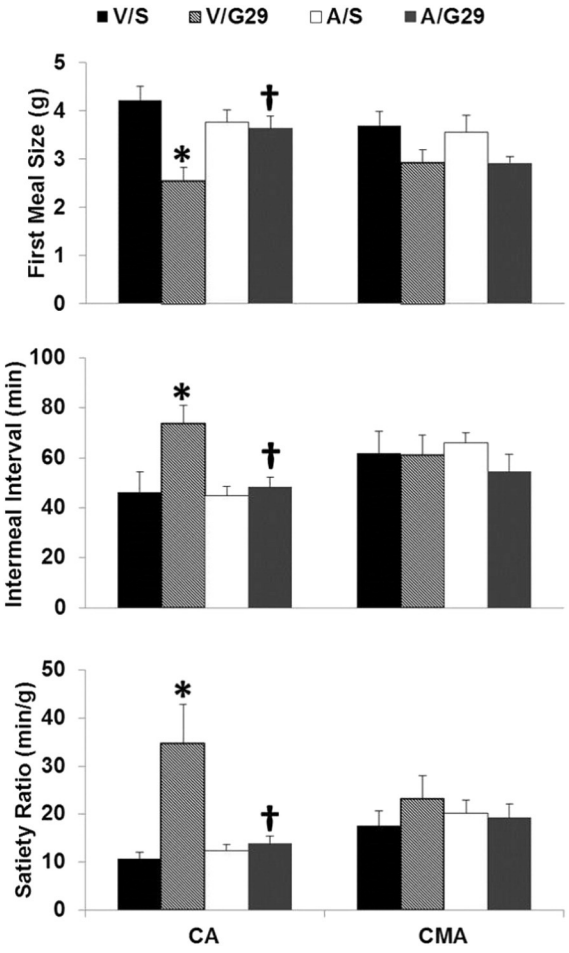

Fig. 1. Effects of the BB_2 receptor antagonist infused in the major arteries of the gut on first meal size, intermeal interval and satiety ratio by gastrin releasing peptide-29. The BB_2 receptor antagonist (BW2258U89, 0.1 mg/kg) or vehicle (saline) were infused in the celiac artery (CA, supplies stomach and upper duodenum) and the cranial mesenteric artery (CMA, supplies small and part of the large intestine) in free feeding rats 10 min prior to the onset of the dark cycle. At the onset of the dark cycle gastrin releasing peptide-29 (GRP-29, 0.5 nmol/kg) or saline vehicle were infused in the same arteries and the size of the first meal (MS, normal rat chow, upper panel), the intermeal interval (IMI, time between first and second meals in min, middle panel) and the satiety ratio (SR, IMI/MS, lower panel) were determined. GRP-29 reduced MS, prolonged the IMI and increased the SR relative to control vehicle (* denotes significance, $p < 0.05$) and the BB_2 receptor antagonist attenuated these responses († denotes significance, $p < 0.05$). V/S, vehicle for antagonist/saline or vehicle for GRP, V/G29, vehicle for the antagonist/GRP-29, A/S, antagonist/saline or vehicle for GRP, A/G29, antagonist/GRP-29