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The BB₂ receptor antagonist BW2258U89 attenuates the feeding responses evoked by exogenous gastrin releasing peptide-29

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Abstract

This confirmatory work is aimed to test that the hypothesis that the gastrin releasing peptide (GRP) receptor – the BB₂ 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 BB₂ 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 BB₂ receptor antagonist BW2258U89 infused in the same artery attenuated these responses. These results suggest that the BB₂ 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.

Keywords

GRP; Meal Size; Intermeal Interval; Satiety Ratio; Celiac Artery

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

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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, BB₁, BB₂ and BB₃, distributed both centrally and peripherally. Gastrin releasing peptide binds the BB₁ receptor less potently than the BB₂ 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 BB₂ receptor antagonist crucial for assigning actions of GRP to the BB₂ receptor. The BB₃ 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; Washington et al., 2011). In addition, reduction of food intake by GRP-10 was attenuated by the BB₂ 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, BB₂, 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 hour 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 spontaneously free feeding rats pretreated with the specific BB₂ 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, BB₂, 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-NH₂2]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 BB₂ 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.

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*.

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; Washington et al., 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.

Statistical Analysis

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

Results

GRP-10

MS, IMI, SR, Latency and Duration—ANOVA for each response revealed no main effects of treatments, routes or treatments and routes interaction.

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

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

GRP-29

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 ($p = 0.001$) (Figure 1, upper panel).

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 ($p=0.008$) (Figure 1, middle panel).

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 ($p=0.005$) and A/GRP-29 infused via the CA attenuated this response ($p=0.001$) (Figure 1, lower panel).

Total Intake—ANOVA revealed a main effect of treatment ($F_{3, 42} = 10.72$, $p=0.003$) $\eta^2=0.32$. Follow-up tests revealed that V/GRP-29 infused via the CA significantly relative to saline control.

Latency to the First Meal—ANOVA revealed a main effect of treatment ($F_{3, 42} = 15.24$, $p=0.001$) $\eta^2=0.43$. Follow-up tests revealed that V/GRP-29 infused via the CA increased the latency of the first meal significantly relative to saline control and A/GRP-29 infused via the CA attenuated this response.

Duration of the first meal—ANOVA revealed a main effect of treatment ($F_{3, 42} = 7.37$, $p=0.001$) $\eta^2=0.23$. Follow-up tests revealed that V/GRP-29 infused via the CA shortened the duration of the first meal significantly relative to saline control and A/GRP-29 infused via the CA attenuated this response.

Number of Meals—ANOVA revealed a main effect of treatment ($F_{3, 42} = 6.76$, $p=0.001$) $\eta^2=0.26$. Follow-up tests revealed that V/GRP-29 did not reduce the number of meals significantly relative to saline control.

Discussion

The current work demonstrated that the BB_2 receptor is necessary for reduction of MS and prolongation of IMI length by exogenous GRP-29. In addition, it confirmed our previous finding (Washington et al., 2014a) that the vascular bed of the celiac artery, stomach and upper duodenum, contains sites of action controlling these responses by the peptide.

The possibility that the BB_2 receptor is necessary for reduction of MS and prolongation of IMI length by endogenous GRP has not been determined by the current study because infusion of the BB_2 receptor antagonist BW2258U89 alone (antagonist / saline, A/S in Figure 1) failed to increase food intake more than in the control group (vehicle / saline, V/S in Figure 1) alone. Such failure may be attributed to the low dose of the antagonist used in this study (0.1 mg/kg) or the time of infusion. Here, we infused the antagonist 10 min prior to the onset of the dark cycle. Normal meals may require more or less time to release endogenous GRP. Therefore, the use of the specific BB_2 receptor antagonist BW2258U89 in the current work documents the necessity of this receptor in reduction of MS and prolongation of the IMI by only exogenous GRP-29.

In our previous work (Washington et al., 2014a) intra-arterial infusion of 0.5 nmol/kg GRP-29 in the CMA reduced MS. In the current study, the same dose of GRP-29 failed to

reduce MS. We cannot provide a clear explanation for this finding. However, the previous study had six rats in every treatment group whereas the current study used eight rats per group. It is possible that by increasing the number of animals per group the effect of the peptide was clearer in the CA compared to the CMA. The tendency for GRP-29 given in the CMA to reduce food intake can be attributed to the wide distribution of the BB₂ receptor in the gut (Sayegh, 2013), which reaches areas that are supplied by the CMA, small and large intestine. Such distribution may have triggered the reduction of MS by GRP-29 given in the CMA. Using smaller doses of the peptide infused in the branches of the CA and CMA e.g. left gastric and cranial pancreaticoduodenal arteries may clarify this result more in the future.

Finally, the fact that we have shown (Reeve et al., 2014) that GRP-29 reduces MS and prolongs the IMI when given by the femoral vein suggests that there are other sites of action regulating the feeding responses by this peptide outside the gastrointestinal tract. Although this possibility has not been tested by the current study, the wide distribution of GRP receptors outside the gut explains this result.

In support of a gastrointestinal site controlling MS and IMI length by GRP-29 we provide the following data. The stomach and upper duodenum contains a high number of BB₂ receptors compared to the remaining portions of the gut and the central nervous system (Sayegh, 2013), and this receptor mediates reduction of food intake by the other mammalian and amphibian forms of GRP e.g., GRP-10, GRP-27 and Bn (Heimbrook et al., 1988; Ladenheim et al., 1996; Merali et al., 1999; Merali et al., 1993; Stratford et al., 1995) (and the current data). Reduction of MS and prolongation of the IMI by GRP-29 requires vagal and splanchnic innervation located in the duodenum (Wright et al., 2012), which are necessary to carry the satiation and the satiety signals of GRP centrally. In addition, we have shown that GRP-29 increases Fos-like immunoreactivity (Fos-LI, a marker for neuronal activation) in the enteric and the dorsal vagal neurons of the hindbrain in areas known to regulate food intake e.g. area postrema and nucleus tractus solitaries (Washington and Sayegh, 2011).

While GRP-10 infused by other routes e.g. intraperitoneal (Washington et al., 2011) and intravenous (Reeve et al., 2014) and in two animal models (Washington et al., 2014b; Washington et al., 2011) reduced MS and prolonged the IMI, it failed to evoke the same responses when given by the CA or the CMA routes (Washington et al., 2014a) (and the current study). This suggests that GRP-10 reduces MS and prolongs the IMI at a non-gastrointestinal site e.g. central and / or endocrine. Such possibilities, however, require further testing. The current work demonstrated that the BB₂ receptor is necessary for reduction of MS and prolongation of the IMI by GRP-29 at sites supplied by the CA.

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Highlights

- Gastrin releasing peptide-29 (GRP-29) is the large molecular form of GRP in the rat
- GRP-29 reduces meal size (MS) and prolongs the intermeal interval (IMI)
- The BB₂ receptor is necessary for MS reduction and IMI prolongation by GRP-29
- The celiac artery supplies sites of action controlling MS and IMI by GRP-29

Figure 1. Effects of the BB₂ 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 BB₂ 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 BB₂ 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

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Figure 2. Effects of the BB₂ receptor antagonist infused in the major arteries of the gut on latency to first meal and duration of the first meal by gastrin releasing peptide-29

The BB₂ 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 latency to the first meal (upper panel) and duration of the first meal size (lower panel) were measured. GRP-29 reduced both first meal latency and duration relative to control vehicle (* denotes significance, p<0.05) and the BB₂ 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