Appetite 109 (2017) 172–181

Contents lists available at ScienceDirect

Appetite

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

Infusion of exogenous cholecystokinin-8, gastrin releasing peptide-29 and their combination reduce body weight in diet-induced obese male rats

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ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form
1 December 2016
Accepted 1 December 2016
Available online 2 December 2016

Keywords:
Cholecystokinin-8
Gastrin releasing Peptide-29
Body weight
Aorta
Diet-induced obese rats

ABSTRACT

We hypothesized that exogenous gastrin releasing peptide-29 (GRP-29), cholecystokinin-8 (CCK-8) and their combination reduce body weight (BW). To test this hypothesis, BW was measured in four groups of diet-induced obese (DIO) male rats infused in the aorta (close to the junctions of the celiac and cranial mesenteric arteries) with saline, CCK-8 (0.5 nmol/kg), GRP-29 (0.5 nmol/kg) and CCK-8+GRP-29 (0.5 nmol/kg each) once daily for a total of 23 days. We found that CCK-8, GRP-29 and CCK-8+GRP-29 reduce BW relative to saline control. In conclusion, CCK-8, GRP-29 and their combination reduce BW in the DIO rat model. If infused near their gastrointestinal sites of action CCK-8, GRP-29 and their combination may have a role in regulating BW.

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1. Introduction

Cholecystokinin (CCK) (Sayegh, 2013b) is a satiety peptide secreted by the I cells of the small intestine, and reduces food intake (Gibbs, Young, & Smith, 1973) by reducing meal size (MS), without altering body weight (BW) (West, Fey, & Woods, 1984). On the other hand, the amphibian skin peptide bombesin (Bn) and its mammalian homologue gastrin releasing peptide-10 (GRP-10) (Sayegh, 2013a), which is secreted by the enteric neurons, reduce MS and lower BW (Ladenheim et al., 2002; West, Williams, Braget, & Woods, 1982).

Data suggest that combining satiety peptides, exogenously, may result in reduction of BW. For example, it has been shown that injections of CCK-8 and leptin reduce BW in mice and rats (Barrachina, Martinez, Wang, Wei, & Tache, 1997; Matson & Ritter, 1999; Matson, Reid, Cannon, & Ritter, 2000). Although this result was not reproducible in the diet-induced obese (DIO) rat model, possibly due to the inability of CCK-8 to overcome leptin resistance in this model (Trevaskis et al., 2010), Trevaskis and colleagues have shown that continuous infusions of stabilized acetylated version of CCK-8, and a synthetic glucagon like peptide-1 receptor agonist, AC3174 (an analogue of exenatide), reduce BW in DIO rats (Trevaskis et al., 2015). As such, CCK-8 may still have a role in regulating BW.

Recently we presented data demonstrating that the sites of action controlling reduction of MS and prolongation of the intermeal interval (IMI) by CCK-8, CCK-33 (Washington, Mhalhal, & Sayegh, 2016b), the only endocrine form of CCK in the rat or CCK-58 (Reeve, Green, Chew, Eysselein, & Keire, 2003) and the non-nutrient releaser of CCK-58, camostat (Lateef, Washington, & Sayegh, 2011; Lateef et al., 2012; Sullivan et al., 2007; Yamamoto, Reeve, Keire, & Green, 2005), are the areas supplied by the celiac artery (CA, supplies stomach and upper duodenum) and the cranial mesenteric artery (CMA, supplies small and part of the large intestine) respectively (Sayegh et al., 2015; Washington, Williams, & Sayegh, 2016). Furthermore, we have shown that the site of action regulating the same feeding responses, MS and IMI length, evoked by GRP-29, the large molecular form of GRP in the rat (Reeve et al., 2014), is the area supplied by the CA (Sayegh et al., 2015; Washington, Aglan, & Sayegh, 2014; Washington, Mhalhal, & Sayegh, 2016a; Washington, Mhalhal, et al., 2016b; Washington et al., 2016). Based on these data, we hypothesized that infusing CCK-8, GRP-29 and their combination reduce BW in the DIO rat model.

To test this hypothesis we infused exogenous CCK-8 and GRP-29 (0.5 nmol/kg each) individually and in combination prior to the

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http://dx.doi.org/10.1016/j.appet.2016.12.001
0195-6663/Published by Elsevier Ltd.

onset of the dark cycle in the aorta, close to the origins of the CA and the CMA, in near spontaneously free feeding DIO male rats once daily for twenty three days and recorded BW, MS (normal rat chow), IMI length, satiety ratio (SR, IMI/MS or amount of food consumed per a unit of time), latency to first meal, length of first meal, total number of meals and total food intake. Our previous work have shown that this dose causes maximum reduction in MS and prolongation of the IMI (Sayegh et al., 2015; Washington, Aglan, et al., 2014; Washington et al., 2016). Consistent with our hypothesis, we found that CCK-8, GRP-29 and CCK-8+GRP-29 reduce BW relative to saline vehicle.

2. Materials and methods

2.1. Animals

The Tuskegee University Animal Care and Use Committee approved the animal protocol for this experiment. Following the arrival to our facility the rats were fed purified rodent diet (D12266B, Research Diets, protein 16.8%kcal, carbohydrates 51.4% kcal and fat 31.8%kcal) for 8 weeks until there was a difference in body weight. After the 8 weeks the animals were maintained on regular rat chow for the remainder of the experiment. Experimental grouping was achieved by body weight rather than random assignment. For this reason body weight was normalized. This indicated that there was no difference in the groups in the pretreatment data, thus selective group assignment had no effect on the results.

Four groups of adult DIO male Sprague Dawley rats (n = 5 rats per group, Charles Rivers, Kingston Facility, NY) with mean starting weight of 641.6 ± 9.07 (group 1), 660 ± 4.39 (group 2), 682.2 ± 9.03 (group 3) and 609.8 ± 7.69 (group 4) 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.

2.2. Vascular catheterization

Each animal had one catheter implanted in the aorta as described previously (Sayegh et al., 2015; Washington, Aglan, et al., 2014; Washington, Mhalhal, et al., 2016b, 2016a,; Washington et al., 2016; Williams et al., 2016). Catheters were inserted in the aorta caudally to cranially (anally to orally) and the tip of each catheter was fixed before the origin of the CMA.

Catheters (Micro-Renathane R-ITC-SP 9.5, Braintree Scientific, Braintree MA) were 24 cm long, the intravascular portion was 0.25 mm OD x 0.12 mm ID and the size of the remaining part was 0.84 mm OD x 0.36 mm ID. Catheterizations were performed using a surgical microscope (Carl Zeiss Opmi 160 12.5×/18B, 1 × 250, Monument, CO). General anesthesia, indicated by the absence of a pedal withdrawal reflex, was achieved with intramuscular injection of 1 ml/kg body weight of a mixture of 5.0 ml of Ketaset [100 mg/kg], 2.5 ml of Rompun® [xylazine 20 mg/kg], Bayer, Shawnee Mission, KS, 1.0 ml of acepromazine maleate® [10 mg/kg], Bayer, Shawnee Mission, KS and 1.5 ml of saline. The abdominal wall was clipped and cleaned with three alternating betadine solution and alcohol swabs. A ventral midline celiotomy was performed.

The aorta was exposed and two temporary ligations, 1 cm a part, were placed passing the origin of the CMA in the aborad direction to prevent bleeding. The aorta was punctured with a sterile 30 gauge needle between the two ligatures and the catheter was threaded into the artery without blocking the entrance of the CMA. The catheter was fixed in place using cyanoacrylate glue, the temporary ligations were removed and the catheter was threaded out of the abdominal cavity subcutaneously, exteriorized between the scapulae and secured with sutures and cyanoacrylate glue.

The muscles of the abdominal wall were closed using a polydioxanone II (4–0) absorbable suture in a simple continuous pattern, and the skin was closed using surgical staples. Postoperative care included Metacam® (Meloxicam® [1.1 mg/kg] Boehringer Ingelheim, St. Joseph, MO) subcutaneously for pain control, and Baytril® (Enrofloxacin® [0.05 ml], Bayer, Shawnee Mission, KS) intramuscularly as an anti-bacterial medication, each given daily for 5 d. Rats were allowed two weeks of recovery time. The criteria for complete recovery following surgery included the absence of clinical signs (e.g., signs of pain, porphyria secretion, cold extremities and lethargy) and the return of food intake to preoperative levels. Catheters were flushed twice daily (0900 h and 1645 h) with 0.3 ml heparinized saline.

2.3. Meal patterns

The BioDAQ E2 Food and Water Intake system detects brief episodes of food intake while minimizing food spillage and hoarding and generates a computerized data stream including times of the initiation of intake activity, the period of the activity, and the weight consumed. The criterion for a meal was consumption of ≥0.2 g, and the criterion for IMI was no feeding activity for ≥15 min (Sayegh et al., 2015; Washington, Aglan, et al., 2014; Washington, Mhalhal, et al., 2016b, 2016a,; Washington et al., 2016; Williams et al., 2016).

2.4. Establishing baseline food intake

Following recovery, rats were habituated to the laboratory environment and experimental design daily for two weeks. At 0900 h the rats were weighed and at 0900 and 1645 h they received a 0.3 ml infusion of heparinized saline in their catheters. At 1700 h,

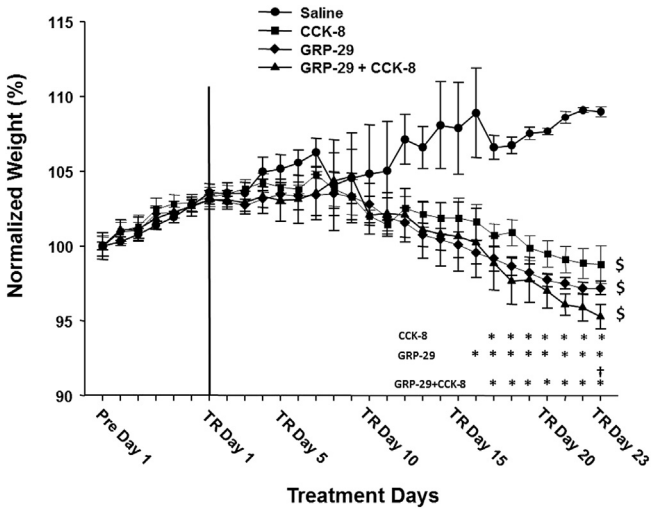

Fig. 1. Effect of cholecystokinin-8, gastrin releasing peptide-29 and combination of the two peptides on body weight. Four groups (n = 5 rats/group) of free feeding, diet-induced obese male rats received daily injection of cholecystokinin-8 (CCK-8, 0.5 nmol/kg), gastrin releasing peptide-29 (GRP-29, 0.5 nmol/kg), CCK-8+GRP-29 (0.05 nmol/kg each) or saline vehicle in the aorta prior to the onset of the dark cycle for twenty three days and body weight (BW) was recorded each morning. Normalized BW increased in all groups ($p = 0.009$) during pretreatment days 1–6. On days 17–23 CCK-8 and CCK-8+GRP-29 reduced BW relative to saline ($p < 0.05$, *). On days 16–23 GRP-29 reduced BW relative to saline ($p < 0.05$ *). On day 23 CCK-8+GRP-29 reduced BW more than CCK-8 ($p < 0.05$, †). CCK-8, GRP-29 and CCK-8+GRP-29 reduced overall BW relative to saline ($p < 0.001$, $).