Physiology & Behavior 104 (2011) 1041–1047

Contents lists available at ScienceDirect

Physiology & Behavior

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

Intragastric infusion of pea-protein hydrolysate reduces test-meal size in rats more than pea protein ☆

Doreen Häberer [a], Maria Tasker [b], Martin Foltz [b], Nori Geary [a], Margriet Westerterp [c], Wolfgang Langhans [a,*]

[a] Physiology and Behaviour Laboratory, Institute of Food, Nutrition and Health, ETH Zurich, 8603 Schwerzenbach, Switzerland
[b] Unilever Research & Development, 3133 AT Vlaardingen, The Netherlands
[c] Department of Human Biology, Maastricht University, 6200 MD Maastricht, The Netherlands

ARTICLE INFO

Article history:
Received 22 December 2010
Received in revised form 1 June 2011
Accepted 3 July 2011

Keywords:
Vagus
Vagotomy
Cholecystokinin
Glucagon-like peptide-1
Glutamate
Eating
Food intake

ABSTRACT

Because protein hydrolysates are digested faster than the corresponding proteins, they may increase or hasten the acute eating-inhibitory effect of protein. Potential mediating mechanisms include accelerated or greater release of satiating gut peptides and activation of metabolic signals that inhibit eating. We tested these hypotheses in adult male rats that were surgically equipped with intragastric (IG) cannulas and adapted to 30-min test meals at dark onset after 14-h food deprivation. Equiosmotic 12 ml loads of saline–urea control (C), 13.6% pea protein (PP), or 13.6% PP hydrolysate (PPH, DSM/DFS, Delft, The Netherlands) solutions were IG infused in 1 min just before test meals. PPH reduced test-meal size compared to C more than PP (-3.8 ± 0.3 g vs. -2.6 ± 0.4 g; $P<0.0001$). Plasma glutamate increased more after PPH than PP ($P<0.0001$). Plasma lactate, alanine, insulin, glucagon, GLP-1 and paracetemol (an index of gastric emptying) all increased similarly, and glucose decreased similarly, after PPH or PP. Finally, PPH still reduced test-meal size more than PP (-4.6 ± 0.3 g vs. -3.1 ± 0.4 g; $P<0.001$) in rats after subdiaphragmatic vagal deafferentation, indicating that abdominal vagal afferents are not necessary for the eating-inhibitory effects of PP and PPH and, by extension, that gut peptides whose satiating effects depend on intact vagal afferents (e.g., CCK and glucagon) do not play crucial roles. Thus, PPH reduced short-term food intake more than PP under our conditions, but the mechanism(s) involved remain unclear.

1. Introduction

Overweight and obesity are pandemic and cause major public health problems because they substantially increase the risk of cardiovascular disease, type 2 diabetes mellitus (T2DM), and of many other chronic diseases (e.g. [1,2]). One common dieting strategy to fight overweight involves eating foods with macronutrient compositions designed to facilitate satiation and body weight loss, such as relatively high-protein diets. Ingestion of relatively high-protein diets is usually associated with decreased energy intake and loss of body weight (see [3–5]), and may even limit weight regain after weight loss [6]. The relatively high-protein diet-induced decrease in energy intake may be related to low palatability (see [4]) or to gastrointestinal and metabolic factors that are associated with protein intake [3–5] and enhance satiation.

Protein given either as a meal or preload has been shown to inhibit eating more than isoenergetic meals or loads of carbohydrates or fats in many acute studies of humans and animals [7–14]. It has been suggested that digestibility may affect the satiating potency of protein [4,15], but the underlying mechanisms of this phenomenon are unknown. We here examined the satiating potency and some potential physiological mechanisms of the acute eating-inhibitory effect of intact or pre-digested protein preloads in rats. Specifically, we compared the effects of intragastric (IG) preloads of intact pea protein (PP) and a pea-protein hydrolysate (PPH, degree of digestion: 18%) on short-term food intake in rats. PP and PPH were chosen for this comparison because in previous studies [16,17] PPH 1) appeared to inhibit eating in humans more potently than other proteins [16] and 2) stimulated the release of the satiating gut peptide cholecystokinin (CCK) from an intestinal secretin tumor cell-line (STC cells) [17]. This *in vitro* study also revealed that some other protein hydrolysates have a direct agonist effect on cholecystokinin-1 (CCK1) receptors [17], which we wanted to avoid. That is, although perhaps unlikely, it is possible that small peptides from IG administered protein hydrolysates reach CCK1 receptors on vagal afferents and enteroendocrine cells in the intestinal wall in sufficient amounts to cause an effect *in vivo*. Because CCK is one of the gut peptides implicated in the satiating effect of protein [18,19], such a direct CCK1 receptor-agonist effect of the protein hydrolysate could mask differential eating-inhibitory effects based on more general features of proteins

☆ Disclosure: This study was supported by a grant from Unilever Research & Development, 3133 AT Vlaardingen, The Netherlands and DSM Food Specialties, 2600 MA Delft, The Netherlands.

* Corresponding author at: Physiology and Behaviour Laboratory, Institute of Food, Nutrition and Health, Schorenstrasse 16, 8603 Schwerzenbach, Switzerland. Tel.: +41 44 655 7420; fax: +41 44 655 7206.
E-mail address: wolfgang-langhans@ethz.ch (W. Langhans).

0031-9384/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2011.07.003

and their corresponding hydrolysates. Our general hypothesis was that because of their greater content of small peptides and free amino acids, protein hydrolysates may cause a more pronounced increase in circulating amino acids, a more rapid release of satiating gut peptides, or other effects leading to early satiation. We tested this hypothesis in adult male Sprague–Dawley rats, surgically equipped with IG cannulas and adapted to a 30-min feeding test (test meal) after 14 h of food deprivation, by 1) comparing the acute eating-inhibitory effects of IG infused PP and PPH, and 2) assessing the effects of PP and PPH on systemic plasma levels of gut peptides, metabolites and gastric emptying. In addition, because many eating-inhibitory signals from the gut are relayed to the brain via vagal afferents [20,21], we also tested whether selective lesion of abdominal vagal afferents altered the acute eating-inhibitory potencies of PP and PPH.

2. Methods

2.1. Animals and housing

Male Sprague–Dawley rats (Charles River, Sulzfeld, Germany) were housed individually in a temperature-controlled (22 ± 2 °C) test room maintained on a 12:12-h light–dark cycle with lights on at 2100 h. Rats were offered tap water and ground chow (No. 3436, Provimi Kliba NAFAG, Kaiseraugst, Switzerland; 54% carbohydrate, 18.5% protein, 4.5% fat and 4.5% fiber; metabolizable energy content, about 13.1 kJ/g) as described below. Rats were adapted to the housing conditions and had ad libitum food and water access for at least 2 weeks before the start of the experiments. All experimental protocols were approved by the Veterinary Office of the Canton of Zurich.

2.2. Surgery

Chronic IG cannulas were surgically implanted in all rats. Rats were anesthetized with a mixture of 100 mg/kg ketamine (Ketasol-100, Gräub, Bern, Switzerland), 5 mg/kg xylazine (Rompun, Bayer, Leverkusen, Germany), and 0.05 mg/kg acepromazine (Prequillan, Arovet, Zollikon, Switzerland). To fabricate cannulas, one end of a 20 cm length of silastic tubing (i.d. 0.762 mm, o.d. 1.60 mm; Ulrich Swiss, St. Gallen, Switzerland) was slipped on a 20-gauge Vacutainer cannula (Becton-Dickenson, Basel, Switzerland) surrounded by folded polypropylene surgical mesh (1.5 × 3 cm; Bard Medica, Oberrieden, Switzerland), and reinforced with another piece of silicon tubing (length: 7 mm, i.d. 1.47 mm, o.d. 1.96 mm). Cannulas were sterilized with ethylene oxide before use. The Vacutainer end was led from a 1.5 cm interscapular incision anteriorly and exteriorized through a stab wound. The cannula was then led subcutaneously to a 3 cm midline laparotomy. A small stab wound was made in the wall of the stomach along the greater curvature, and the cannula was inserted 2 mm into the stomach and fixed in place with a purse-string suture. After at least 11 days of recovery, rats were adapted to the feeding schedule described below for 7 days before experiments began.

2.3. IG infusions and test procedures

Pea protein (PP) and the corresponding hydrolysate (PPH, degree of hydrolysis = 18%) were gifts from DSM Food Specialties (Delft, The Netherlands). We infused 12 ml of 13.6% PP or 13.6% PPH, each dissolved in 0.9% saline, or an equiosmotic 0.9% saline–urea control solution (C) at lights off. Infusions were done in <1 min/rat. Urea was added to the protein and control solutions to bring them to the same osmolarity as the hydrolysates (800 mOsmol/L for Experiments 1 and 2, and 550 mOsmol/L for Experiments 3 and 4). Cannulas were flushed with 1.5 ml 0.9% saline after each infusion.

IG infusions were done just before dark onset, after 14 h of food deprivation. Immediately after infusions, rats were offered food for 30 min (test meal). Food was then removed for 4.5 h and then presented again for 5 h. This design was used 1) to dissociate acute from possible longer-term effects of PP and PPH and 2) to mimic a situation in which humans take a pre-meal protein preload in order to facilitate satiation. Food intake during both periods was measured by weighing (±0.1 g) the food cups and spillage collected on papers spread beneath the cages. The sum of the intakes during the two periods is subsequently referred to as daily food intake.

2.4. Experiment 1: effects of PP and PPH on food intake

The satiating effects of PP and PPH were tested in a between-subject trial. Twenty-four rats weighing 349 ± 8 g (mean ± SE) at experiment onset were divided into three groups (urea–saline = control, PP, and PPH) that each received one 12 ml IG infusion on the test day. The treatment groups were roughly matched according to body weight and test-meal size during the last adaptation day prior to each trial.

2.5. Experiment 2: effects of PP and PPH on blood parameters

Ten rats weighing 304 ± 6 g at experiment onset were prepared with both an IG cannula and a jugular vein catheter, which was implanted according to the method described by Steffens [22], with some modifications [23,24]. The distal end of the catheter was inserted into the maxillary vein 1 mm above the junction with the facial and linguofacial veins and advanced until its tip was located inside the right atrium. We supposed the catheter was correctly placed if we were able to withdraw blood from it and if a pulse was evident in the blood in the catheter. The catheter was kept in place by two ligatures, and its protruding end was attached to the headpiece of the gastric cannula.

After recovery from surgery, rats were adapted as previously. We then IG infused PP, PPH or C in a within-subject crossover design, with two intervening days between treatments during which rats were kept on the restricted-feeding schedule. On test days rats did not have access to food after intragastric infusions, and 500 µl blood samples were taken via the jugular vein catheters just before IG infusions and 3, 15 and 30 min afterwards.

Blood was mixed with EDTA (1.6 mg/ml blood), kept on ice for <10 min, and centrifuged for 8 min at 5000 × *g* and 4 °C. Plasma was aliquoted and stored at −70 °C. Plasma glucose, lactate and alanine, and free glutamate in the plasma and the infusate were analyzed by standard enzymatic methods adapted for the Cobas Mira autoanalyzer (Roche Diagnostics, Basel, Switzerland) [25]. Plasma insulin, glucagon and glucagon-like peptide-1 (GLP-1) were determined by LINCOplex (Linco, St. Charles, MO, USA). In order to measure gastric emptying, 1.7% paracetamol (acetaminophen, Sigma Chemical Co., St. Louis, MO, USA) was added to all infusates [26,27] and was assayed in plasma enzymatically (Cambridge Life Sciences, Cambs, United Kingdom).

2.6. Experiment 3: role of subdiaphragmatic vagal afferents in the eating-inhibitory effects of PP and PPH

Subdiaphragmatic vagal deafferentation (SDA), which consists of transection of the left vagal afferent rootlets at the brain stem and of the left (or dorsal) subdiaphragmatic esophageal vagal trunk [28] was done as described previously [29]. During sham operations (Sham), the left vagal afferent rootlets and subdiaphragmatic esophageal vagal trunk were exposed but left untouched. Sham and SDA rats weighed 414 ± 15 and 422 ± 13 g, respectively, when the experiment started. Twelve milliliters of 13.6% PP, PPH or control solution were IG infused at lights off, using a within-subject design within surgical groups, and FI was measured as described above.

SDA were verified histologically and functionally [29]. Four micrograms per kilogram of CCK-8 (Bachem, Bubendorf, Switzerland) in phosphate buffered saline (PBS) or vehicle was intraperitoneally