Physiology & Behavior xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Physiology & Behavior

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

High fat diet induced changes in gastric vagal afferent response to adiponectin

Stephen J. Kentish [a,b,*], Kyle Ratcliff [a], Hui Li [a], Gary A. Wittert [a,b,c], Amanda J. Page [a,b,c]

[a] Vagal Afferent Research Group, Centre for Nutrition and Gastrointestinal Disease, Discipline of Medicine, University of Adelaide, Frome Road, Adelaide, SA 5005, Australia
[b] Nutrition and Metabolism, South Australian Health and Medical Research Institute, North Terrace, SA 5000, Australia
[c] Royal Adelaide Hospital, North Terrace, Adelaide, SA 5000, Australia

HIGHLIGHTS

- Adiponectin is expressed in the stomach.
- Adiponectin receptors are present in mucosal and muscular gastric vagal afferents.
- Adiponectin inhibits the mechanosensitivity of tension gastric vagal afferents.
- In obese mice adiponectin potentiates mucosal vagal afferent mechanosensitivity.

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 28 May 2015
Accepted 10 June 2015
Available online xxxx

Keywords:
Vagal afferents
Obesity
Adiponectin

ABSTRACT

Food intake is regulated by vagal afferent signals from the stomach. Adiponectin, secreted primarily from adipocytes, also has a role in regulating food intake. However, the involvement of vagal afferents in this effect remains to be established. We aimed to determine if adiponectin can modulate gastric vagal afferent (GVA) satiety signals and further whether this is altered in high fat diet (HFD)-induced obesity.

Female C57BL/6J mice were fed either a standard laboratory diet (SLD) or a HFD for 12 weeks. Plasma adiponectin levels were assayed, and the expression of adiponectin in the gastric mucosa was assessed using real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR). The location of adiponectin protein within the gastric mucosa was determined by immunohistochemistry. To evaluate the direct effect of adiponectin on vagal afferent endings we determined adiponectin receptor expression in whole nodose ganglia (NDG) and also specifically in GVA neurons using retrograde tracing and qRT-PCR. An in vitro preparation was used to determine the effect of adiponectin on GVA response to mechanical stimulation.

HFD mice exhibited an increased body weight and adiposity and showed delayed gastric emptying relative to SLD mice. Plasma adiponectin levels were not significantly different in HFD compared to SLD mice. Adiponectin mRNA was detected in the gastric mucosa of both SLD and HFD mice and presence of protein was confirmed immunohistochemically by the detection of adiponectin immunoreactive cells in the mucosal layer of the stomach. Adiponectin receptor 1 (ADIPOR1) and 2 (ADIPOR2) mRNA was present in both the SLD and HFD whole NDG and also specifically traced gastric mucosal and muscular neurons. There was a reduction in ADIPOR1 mRNA in the mucosal afferents of the HFD mice relative to the SLD mice. In HFD mice adiponectin potentiated gastric mucosal afferent responses to mucosal stroking, an effect not observed in SLD mice. Adiponectin reduced the responses of tension receptors to circular stretch to a similar extent in both SLD and HFD mice.

In conclusion, adiponectin modulates GVA satiety signals. This modulatory effect is altered in HFD-induced obesity. It remains to be conclusively determined whether this modulation is involved in the regulation of food intake and what the whole animal phenotypic consequence is.

1. Introduction

It is well established that vagal afferent signals generated in the gastrointestinal tract are able to influence food intake [1,2]. In the stomach, two mechanosensitive gastric vagal afferent (GVA) receptor subtypes, tension and mucosal receptors, have been identified based on their response to mechanical stimuli [3]. Although the role of gastric tension

* Corresponding author at: Centre for Nutrition and Gastrointestinal Disease, Discipline of Medicine, University of Adelaide, Level 7 South Australian Health and Medical Research Institute, North Terrace, Adelaide, SA 5000, Australia.
E-mail address: stephen.kentish@adelaide.edu.au (S.J. Kentish).

http://dx.doi.org/10.1016/j.physbeh.2015.06.016
0031-9384/

receptors in signaling distension to trigger satiety and accommodation has been well characterized [4], the role of mucosal receptors is less well understood. Andrews and Sanger suggested that mucosal receptors play a role in detecting chemical stimuli and generating sensations of nausea and triggering vomiting [5]. Mucosal receptors have also been proposed to be important in measuring food particle size and therefore digestion [6]. Particle size is a factor which influences the rate of gastric emptying, which relates inversely to satiety [7,8]. The mechanosensitivity of GVAs is modulated by peptides known to affect satiety and feeding behavior, released either locally in the gut or into the circulation [9–11]. Thus, vagal endings within the stomach wall are in an ideal position to detect not only mechanical stimuli but also locally released peptides/hormones from enteroendocrine cells in the gastric mucosa [12].

GVA satiety signals are influenced by nutritional status. For example, the sensitivity of tension sensitive GVA mechanoreceptors is reduced with fasting or chronic high fat diet (HFD) consumption [13]. Furthermore, the modulatory effect of appetite regulating hormones on GVAs changes with short-term food withdrawal or long-term HFD feeding [12].

Adiponectin is an adipokine that is abundant in the circulation, representing up to 0.05% of total circulating protein [14]. Adiponectin has a primary insulin sensitizing action but also has beneficial effects on metabolism and atherosclerosis [15–19]. Similar to other adipokines, adiponectin has been shown to affect satiety both centrally and peripherally, although there is a lack of consensus in rodent studies regarding the ability of adiponectin to regulate food intake. An intracerebroventricular (ICV) infusion of adiponectin can reduce body weight by either decreasing food intake and/or increasing energy expenditure [20,21]. However, in contrast, a central effect has been observed to increase food intake and cause weight gain by activation of adiponectin receptors in the hypothalamus [22]. Peripherally, overexpression of adiponectin in rats reduced food intake [23]. In contrast, adiponectin knockout mice have normal feeding behavior when fed standard chow [22,24], but reduced food intake in response to a high fat diet compared to wild type mice [22]. The effect of adiponectin on vagal afferents, has to date remained unexplored. Furthermore, the stomach has also been shown express leptin which acts in a paracrine manner on vagal afferents [25] but whether this also applies to adiponectin has not hitherto been explored.

Two receptors for adiponectin have been identified, adiponectin receptor 1 (ADIPOR1) and adiponectin receptor 2 (ADIPOR2) [26], which appear to have opposite effects. Deletion of ADIPOR1 increases adiposity and decreases both glucose tolerance and locomotor activity, whilst deletion of ADIPOR2 results in a lean phenotype with increased locomotor activity and resistance to HFD-induced obesity [27]. The expression of adiponectin receptors is linked to metabolic status with abundance inversely proportional to circulating insulin levels [28]. The nodose ganglia (NDG) containing the cell bodies of vagal afferents incorporate a variety of receptors for peptides with food intake modulatory effects such as leptin, ghrelin and cholecystokinin [29]. The presence of adiponectin receptors in vagal afferents has not previously been reported and there are also no prior reports, as far as can be determined, as to whether a peripheral vagal pathway exists for adiponectin and whether any potential GVA signaling is disrupted in obesity where circulating adiponectin concentrations have been suggested to be decreased.

The aim of the present study was to determine the expression of adiponectin receptors in the NDG, whether adiponectin is able to signal through GVAs and use an obese mouse model to determine whether such signals are disrupted in HFD induced obesity.

2. Materials and methods

2.1. Ethical approval

All experimental protocols were approved by the animal ethics committees of the Institute of Medical and Veterinary Science and University of Adelaide and were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2. High fat diet model

Thirty-two, 7 week old female C57BL/6J mice (Animal Resource Centre, Perth, Australia) were acclimatized for 1 week before being randomly divided into two equal groups and fed either a high fat diet (HFD) comprising 60%, 20%, and 20% of energy from fat, protein, and carbohydrate (Adapted from Research Diets Inc., New Brunswick, USA) or a standard laboratory diet (SLD) comprising 12%, 23%, and 65% of energy from fat, protein and carbohydrate (Specialty Feeds, Glen Forest, Western Australia) for 12 weeks (N = 16/diet). Four mice were housed per cage and were kept under 12 h light:dark cycles with ad libitum access to food and water.

2.3. Quantification of plasma adiponectin and insulin

Plasma samples extracted from the blood collected from the abdominal aorta during euthanasia were analyzed for circulating insulin and adiponectin using a Millipore Insulin ELISA kit (EZRMI-13K) and Millipore Adiponectin ELISA kit (EZMADP-60K) respectively as per the manufacturer's instructions (Millipore, Massachusetts, USA). Quantification was measured at 460 and 595 nm. The adiponectin assay has a detection threshold of 0.2 ng/mL with an intra-assay variation of 5.75% and an inter-assay variation of 5.97%. The insulin assay kit has a detection threshold of 0.1 ng/mL, with an intra-assay variation of 3.73% and an inter-assay variation of 6.03%.

2.4. Gastric emptying

Gastric emptying was measured using a calibrated solid egg meal as previously described [30], the week prior to euthanasia for the GVA recordings. Briefly, after an overnight fast mice from both diet groups (N = 12/diet) were given 0.1 g of baked egg yolk containing 1 μg mL^{-1} of [^{13}C]-labeled octanoic acid (99% enrichment, Cambridge Isotope Laboratories, MA, USA) to consume within 1 min. Breath samples were collected at regular intervals for solid meal emptying (0–150 min) and analyzed for [$^{13}CO_2$] content with an isotope ratio mass spectrometer (ABCA 20/20 Europa Scientific, Crewe, UK). The [$^{13}CO_2$] excretion data was analyzed by nonlinear regression analysis for curve fitting and for calculation of gastric half-emptying time (t½).

2.5. Retrograde tracing

Cell bodies of GVAs innervating specific stomach layers were identified using differential tracing from the stomach as previously documented [31].

2.5.1. Gastric muscle

SLD (n = 8) and HFD (n = 8) mice were anesthetized with isoflurane (1–1.5% in oxygen), a laparotomy performed, and an Alexa Fluor® 555 conjugate of cholera toxin β-subunit (CTB-AF555 (0.5%); Invitrogen, Life Technologies, Mulgrave, Australia) injected subserosal into the muscularis externa of the proximal stomach using a 30 gauge Hamilton syringe. Multiple equally spaced injections of 2 μL were made parallel to and 1–2 mm from the lesser curvature on both dorsal and ventral surfaces of the stomach (total volume 10 μL). The injection sites were dried with a cotton tip to ensure no spillage of tracer, the laparotomy incision was closed, and antibiotic (Baytrill 10 mg 50 μL^{-1}) and analgesic (Butorphenol; 5 mg kg^{-1}) were administered.

2.5.2. Gastric mucosa

SLD (n = 8) and HFD (n = 8) mice were anesthetized with isoflurane (1–1.5% in oxygen), a laparotomy performed and a mucolytic

Please cite this article as: S.J. Kentish, et al., High fat diet induced changes in gastric vagal afferent response to adiponectin, Physiol Behav (2015), http://dx.doi.org/10.1016/j.physbeh.2015.06.016