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Apelin modulates murine gastric vagal afferent mechanosensitivity

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A B S T R A C T

Gastric vagal afferents play an important role in the peripheral control of food intake. Apelin, a central appetite regulating hormone, is also abundantly released from the stomach. Whether apelin modulates gastric vagal afferent signalling is unknown. We aimed to determine whether apelin modulates gastric vagal afferent signalling under different states of nutrition.

Female C57BL/6 mice were fed either a standard laboratory diet (SLD) or a high fat diet (HFD) for 12 weeks. An in vitro gastric vagal afferent preparation was used to determine the effect of apelin on gastric vagal afferent mechanosensitivity in SLD mice, fed ad libitum or fasted overnight, and HFD mice. To determine the signalling pathway of apelin via gastric vagal afferents, we determined the expression of apelin receptor (APJ receptor) in the gastric mucosa, the whole nodose ganglion and in gastric vagal afferent neurons innervating the stomach using retrograde tracing and real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR). The location of apelin and APJ receptor within the gastric mucosa was determined by immunohistochemistry. Expression of apelin and APJ receptor mRNA in gastric mucosa was determined using qRT-PCR.

Apelin inhibited the response of gastric mucosal vagal afferents to mucosal stroking in fasted SLD mice, but not in mice fed ad libitum a SLD or HFD. Apelin inhibited the response of gastric tension sensitive afferents to circular stretch in SLD mice fed ad libitum or fasted, an effect not observed in HFD mice. APJ receptor mRNA was detected in the gastric mucosa and whole nodose ganglion, but not specifically in gastric vagal afferents neurons. In the gastric mucosa, APJ receptor immunoreactive cells were co-localised or closely associated with apelin containing cells and co-localised with serotonin, gastrin, histamine and gastric intrinsic factor containing cells.

In conclusion, apelin modulates gastric vagal afferent signalling in a nutritional status dependent manner. Further, apelin modulates gastric vagal afferents through an indirect pathway, possibly through the release of hormones/peptides from the gastric mucosa.

Keywords
Apelin
Food restriction
High fat diet
Obesity
Vagal afferents

1. Introduction

Gastrointestinal vagal afferents play an important role in the regulation of food intake and gut function [1, 2]. These sensory nerves sense mechanical and chemical stimulation within the gastrointestinal tract and send signals to the central nervous system (CNS) to modulate feeding behaviour. In the stomach gastric vagal tension sensitive receptors are located within the gastric muscular layers. They sense the distension of the gastric wall and send signals to the CNS to induce satiation and trigger reflexes to control gastric function [3, 4]. Mucosal receptors, located within the mucosal layer, are activated by the mechanical contact of food particles. It has been suggested, mucosal receptors are responsible for discrimination of food particle size providing negative feedback on the control of gastric emptying [5, 6].

Vagal afferent signalling can be altered in both physiological and pathological conditions. For example in mice, gastric tension receptor responses to stretch are reduced after an overnight fast [7]; probably an adaptive response to reduce satiety signals and facilitate food intake. In comparison to control mice the response of gastric tension receptors to stretch stimuli is reduced in high fat diet (HFD)-induced obese mice [7]. This dampened satiety signal may contribute to the disrupted feeding

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behaviour observed in obesity.

Numerous studies have demonstrated hormonal modulation of gastric vagal afferent signalling. Several appetite regulatory hormones, besides their central roles in the regulation of food intake and digestion, are also released from the stomach and can locally modulate gastric vagal afferents, constituting a peripheral mechanism for the regulation of food intake. For example, leptin, ghrelin and neuropeptide W, are released from the gastric mucosa and can modulate the response of gastric vagal afferents to mechanical stimulation [8–10]. Furthermore, their modulatory effects are altered after short-term food deprivation and in HFD-induced obesity [7, 8, 10].

Apelin is the endogenous ligand for the G protein-coupled apelin receptor (APJ receptor) [11]. Evidence suggests that apelin acts centrally to regulate feeding behaviour and energy balance. Apelin and APJ receptor expression have been located in the hypothalamus, including the arcuate nucleus and the paraventricular nucleus, brain regions with major appetite regulatory roles [12–14]. In mice, acute intracerebroventricular (i.c.v) injection of apelin reduced [15, 16], increased [17] or had no effect on food intake [18]. In rats, chronic i.c.v. injection of apelin induced an increase in food intake and body weight [19]. Apelin may also play a role in the peripheral modulation of food intake. Apelin is present in the gastrointestinal tract with the highest levels observed in the stomach [20], where it has been reported to inhibit gastric acid via the modulation of histamine production and stimulating proliferation of gastric cells [20, 21]. In a murine enteroendocrine cell line (STC-1), cholecystokinin (CCK) secretion is upregulated by apelin [20]. It remains to be determined whether apelin modulates gastric vagal afferents signalling.

This study aimed to determine whether apelin can directly or indirectly modulate the response of gastric vagal afferents to mechanical stimulation. Further, this study will determine whether the modulatory effects of apelin are dependent on nutritional status by examining the effects of apelin after overnight food deprivation and after chronic consumption of a HFD.

2. Materials and methods

2.1. Ethical approval

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

2.2. Mice

Seven-week-old female C57BL/6 mice were group housed with free access to water and food. After acclimatisation for 1 week, mice were given a standard laboratory diet (SLD) or a HFD for 12 weeks. SLD mice were given a diet comprising 12%, 23% and 65% of energy from fat, protein and carbohydrate (Specialty Feeds, Glen Forest, Western Australia), and HFD mice were given a diet comprising 60%, 20% and 20% of energy from fat, protein and carbohydrate (Adapted from Research Diets Inc., New Brunswick, USA). After the completion of the 12 week diet period, a subgroup of SLD mice were fed ad libitum (SLD ad libitum), while the other subgroup of SLD mice were fasted overnight for 17 h prior to the experiment (SLD fasted).

2.3. In vitro mouse gastro-oesophageal vagal afferent preparation

This preparation has been described in detail previously [22, 23]. Briefly, C57BL/6 mice from SLD ad libitum, SLD fasted or HFD group were culled via CO_2 inhalation at 9 am on the day of the experiment. The thorax was opened by midline incision, and the stomach and oesophagus with attached vagal nerves were removed and placed into a modified Krebs solution containing (mM): NaCl 118.1, KCl 4.7, $NaHCO_3$ 25.1, NaH_2PO_4 1.3, $MgSO_4.7H_2O$ 1.2, $CaCl_2$ 1.5, citric acid 1.0 and glucose 11.1, bubbled with 95% O_2–5% CO_2 at 4 °C during dissection to prevent metabolic degradation. Nifedipine (1 μM) was also added to the Krebs superfusate to prevent smooth muscle contraction. After further dissection, the preparation was opened out longitudinally along the oesophagus and the greater curvature of the stomach. The preparation was then placed mucosal side up in an organ bath. The vagus nerves were extended into a second chamber where they were rested on a glass plate and bathed in paraffin oil. Under a dissecting microscope the nerve sheath was gently peeled back to expose the nerve trunk. Using fine forceps, nerve fibres were teased apart into 8–14 bundles then, one by one, the small bundles were placed onto a platinum recording electrode. A reference electrode rested on the glass plate in a small pool of Krebs' solution.

2.4. Characterization of gastric vagal afferent properties

Two types of mechanosensitive afferent fibre were studied, those responding to mucosal stroking but not circular tension (mucosal receptors) and those responding to mucosal stroking and circular tension (tension receptors) as reported previously.

Briefly, the location of receptive fields of gastric vagal afferent was identified by mechanical stimulation throughout the preparation with a brush. The sub-type of gastric vagal afferent was then further determined through applying a more refined stroking or stretch stimuli. For mucosal stimulation calibrated von Frey hairs (10-1000 mg) were used to stroke over the receptive field at a rate of about 5 mm/s. The mechanical response to circular tension was determined using a cantilever and hook system. A threaded hook was attached to the stomach at a point adjacent to the receptive field. The other end of the threaded hook was attached to a cantilever system via a pully close to the preparation. Weights (1-5 g) were placed on the opposite end of the cantilever system for 1 min. Single units were discriminated by action potential shape, duration and amplitude using Spike 2 software (Cambridge Electronic Design, UK).

2.5. Effect of apelin on gastric vagal afferent mechanosensitivity

After the baseline mechanosensitivity of a gastric vagal afferent had been established the effect of apelin on gastric vagal afferent mechanosensitivity was determined. Apelin (1 nM) was added to the Krebs, superfused over the gastric tissue and allowed to equilibrate for 20 min to ensure penetration of all layers of the gastric tissue before vagal afferent mechanosensitivity was redetermined. This was repeated for the higher concentrations of Apelin (3 and 10 nM). Stock solution of apelin-13 (#2420, Tocris, VIC, Australia) were kept frozen (−80°C) and diluted to final concentration in Krebs solution on the day of the experiment. Time control experiments were performed in which there was no significant change in mucosal or tension receptor responses to mechanical stimulation over a comparable duration.

2.6. Data recording and analysis

Afferent impulses were amplified with a biological amplifier (DAM 50, World Precision Instruments, Sarasota, FL, USA), and filtered (band-pass filter 932, CWE, Ardmore, PA, USA). Response of units were analysed by use of Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

2.7. Retrograde tracing

Cell bodies of gastric vagal afferents innervating specific stomach layers were identified using differential tracing from mouse stomach as previously documented [10].