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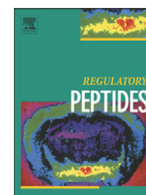

Gastrin release: Antrum microdialysis reveals a complex neural control

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ABSTRACT

We used microdialysis to monitor local gastrin release in response to food, acid blockade and acute vagal excitation. For the first time, gastrin release has been monitored continuously in intact conscious rats in a physiologically relevant experimental setting in a fashion that minimizes confounding systemic effects. Microdialysis probes were placed in the submucosa on either side of the antrum, 3 days before the experiments. The concentration of gastrin in the antral submucosal compartment was about 20 times higher than in the microdialysate and estimated to be 5–10 times higher than in serum regardless of the prandial state. The rats were conscious during microdialysis except when subjected to electrical vagal stimulation. Acid blockade (omeprazole treatment of freely fed rats for 4 days), or bilateral sectioning of the abdominal vagal trunks (fasted, 3 days post-op.), raised the gastrin concentration in blood as well as microdialysate. The high gastrin concentration following omeprazole treatment was not affected by vagotomy. Vagal excitation stimulated the G cells: electrical vagal stimulation and pylorus ligation (fasted rats) raised the gastrin concentration transiently in both serum and microdialysate. Food intake induced a 2- to 3-fold increase in serum gastrin, while gastrin in antral microdialysate increased 10- to 15-fold. In unilaterally vagotomized rats (fasted, 3 days post-op.), food evoked a prompt peak gastrin release followed by a gradual decline on the intact side. On the vagotomized side of the antrum, the peak response seemed to be reduced while the microdialysate gastrin concentration remained elevated. Thus, unilateral vagotomy surprisingly raised the integrated gastrin response to food on the denervated side compared to the intact side, indicating that vagotomy suppresses an inhibitory as well as a stimulating effect on the G cells. While local infusion of atropine was without effect, infusion of the neuronal blocker tetrodotoxin (TTX) (which had no effect on basal gastrin) virtually abolished the food-evoked gastrin response and lowered the high microdialysate gastrin concentration in omeprazole-treated rats by 65%. We conclude that activated gastrin release, unlike basal gastrin release, is highly dependent on a neural input: 1) Vagal excitation has a transient stimulating effect on the G cells. The transient nature of the response suggests that the vagus has not only a prompt stimulatory but also a slow inhibitory effect on gastrin release. 2) Although vagal denervation did not affect the gastrin response to anacidity, the TTX experiments revealed that both food-evoked and anacidity-evoked gastrin release depends on neural input.

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1. Introduction

Gastrin from G cells in the antrum is the main stimulus of gastric acid secretion. Gastrin stimulates the ECL cells in the oxyntic mucosa [1,2] to mobilize histamine [3,4], which in turn stimulates the parietal cells to produce hydrochloric acid [4–8]. Gastrin is released in response to a variety of food-related stimuli, such as elevated luminal pH [9,10], intraluminal peptides, amino acids and amines [11,12], and distension of the stomach [13–15]. In addition to this, agents released from endocrine cells in the vicinity of the G cells (for example, somatostatin released from D cells) [16–18], as well as hormones reaching the antrum via the circulation [19–21], contribute to the control of gastrin release. Further, G-cell secretion is regulated by the enteric nervous system and the autonomic nervous system (via transmitters such as acetylcholine and gastrin-releasing peptide) [19,22–25].

Gastrin release has been the subject of numerous studies in the past using either *in vivo* or *in vitro* techniques. The drawback of the *in vivo* methods is that it is usually difficult to decide whether the G-cell response reflects a direct effect of the experimental intervention or occurs as a consequence of confounding systemic effects. *In vitro* methods, such as isolated stomachs [4,19,21,22], antral sheets [26] or isolated G cells [12,25,27,28] are generally thought to allow more direct studies of the G cell. However, *in vitro* methods are inherently "unphysiological" in that nervous and hormonal circuits that may affect the ability of the G cells to respond to stimuli are not operative.

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The method of microdialysis was first applied to the rat gastric submucosa by Bunnett et al. [29]. By using a similar approach, we have developed a protocol for the study of histamine mobilization from the ECL cells in the acid-producing part of the rat stomach with the use of microdialysis probes placed in the gastric submucosa [30–32]. The advantage of the microdialysis technique, as compared to measuring circulating concentrations of a substance, lies in the fact that the microdialysis probe operates as an artificial blood vessel. This allows the continuous monitoring of substances in the extracellular fluid in tissues of intact conscious animals [33,34]. Also, by reverse microdialysis agents can be delivered locally in the gastric submucosa via the probe, enabling stimulation or inhibition of the target cells with less risk of causing systemic effects [35]. Hence, the microdialysis technique has the advantage over *in vitro* methods and over other *in vivo* methods in that specific cell populations can be studied in whole animals under physiologically relevant experimental conditions. The usefulness of the microdialysis technique is limited mainly by the sensitivity of the monitoring assay and the ability of compounds to pass the dialysis membrane.

Measurement of gastrin in serum following different kinds of experimental manipulations is a conventional approach to monitor gastrin release *in vivo*. However, these manipulations may interfere with the activity of the G cells. For instance, effects on acid secretion, on the central and peripheral nervous systems or on endocrine cells other than G cells may influence the G cells in an indirect manner, complicating the interpretation of the results. Another complicating factor in the *in vivo* situation is that following its release, gastrin is promptly being distributed, and diluted, in the blood stream, making it difficult to achieve precision in monitoring the process of gastrin release by measuring the serum gastrin concentration. The vascularly perfused rat stomach is an alternative experimental model, which reduces the impact of confounding systemic factors and makes it possible to monitor (even control) luminal acidity. However, there are problems: 1) The stomach is not in continuity with the remainder of the digestive tract. 2) Circulation is maintained artificially by perfusion with a salt solution. 3) All inputs from extra-gastric neurocrine and endocrine systems have been eliminated.

The present study addresses the control of gastrin release from the G cells in the antrum. The primary purpose was to develop a protocol for studying gastrin release in intact, conscious rats in physiologically relevant experimental settings by the use of antral submucosal microdialysis and to compare measurement of gastrin in blood and microdialysate following a series of treatments assumed to influence gastrin release. The secondary purpose of the study was to explore the significance of nervous control of the G cells in relation to basal and stimulated gastrin release.

2. Materials and methods

2.1. Ethical approval

The studies were approved by the local Animal Welfare Committee of Lund/Malmö.

2.2. Chemicals

The proton pump inhibitor omeprazole was a gift from AstraZeneca (Mölndal, Sweden). Omeprazole was dissolved in 0.25% Methocel (methyl cellulose) (Dow Corning, Midland, MI, USA) and administered once daily (400 µmol kg^{-1} day^{-1}) between 7:00 and 9:00 a.m. for 4 days by oral gavage. This treatment is known to block acid secretion [36]. The last dose was administered in the morning on the day of the experiment. Saline (0.9% NaCl) was used for perfusion via the microdialysis probes.

Tetrodotoxin (TTX) (Alomone Labs, Jerusalem, Israel), a drug known to block nervous conductance [37], and atropine sulphate (Sigma, St. Louis, MO, USA), a muscarinic receptor blocker, were dissolved in saline for perfusion via the microdialysis probe.

2.3. Animals

130 male and 11 female (as specified) Sprague–Dawley rats (250–300 g) were kept at a 12-h light and 12-h dark cycle in plastic cages (2–3 in each cage) with free access to standard rat food pellets (B & K Universal, Sollentuna, Sweden) and tap water. When the rats were to be fasted, they were housed in individual wire mesh bottom cages with free access to water overnight for 24 h before the experiments. In experiments involving refeeding they were offered standard rat pellets and tap water for 3 h. Microdialysis experiments were performed on conscious animals, except those experiments that involved electrical vagal stimulation (see below). During sampling of microdialysate they were kept in Bollman-type restraining cages. Starting 1 week prior to the experiments the rats were familiarized with the Bollman cages by daily training for 1–2 h. Food and water was available during the training sessions. Blood samples for measurement of gastrin in serum were drawn from the tip of the tail, usually during the equilibration period (just before the start of microdialysate sampling) and at the termination of each experiment (after collecting the last microdialysate sample). Each rat was killed by exsanguination from the abdominal aorta following an overdose of chloral hydrate intraperitoneally.

2.4. Surgery

2.4.1. Anaesthesia

If not otherwise stated, surgery, including implantation of the microdialysis probe (see below), was performed under chloral hydrate anaesthesia (300 mg kg^{-1} intraperitoneally), 3 days prior to the microdialysis experiments. Surgery was performed on freely fed rats. Buprenorphine (Temgesic®, Schering-Plough, NJ, USA) was given subcutaneously (0.02 mg kg^{-1}) at the time of surgery to alleviate postoperative pain. No mortality was associated with the surgery. No antibiotics were used. Experiments involving electrical stimulation of the vagus (see Experimental design) were performed on rats anaesthetized with fluanisone/fentanyl/midazolam (15/0.5/7.5 mg kg^{-1}, intraperitoneally), since this anaesthesia has less inhibitory effect on gastric endocrine cells than chloral hydrate [31].

2.4.2. Unilateral vagal denervation

Unilateral vagotomy was performed by opening the abdominal cavity by a midline incision and by exposing the ventral vagus nerve along the oesophagus below the diaphragm before cutting it as close as possible to the stomach. The dorsal vagus nerve was left intact. The rats were fitted with microdialysis probes at the same time (see below).

2.4.3. Bilateral vagal denervation

Total abdominal vagotomy was achieved by cutting both vagal trunks immediately below the diaphragm. A pyloroplasty was performed at the same time to prevent gastric dilation [38], and the rats were also fitted with microdialysis probes in the dorsal part of the antrum (see below). We have shown previously that pre- and postprandial serum gastrin concentrations in rats subjected to pyloroplasty do not differ from unoperated rats [39]. The effectiveness of the bilateral vagal denervation was verified by hypergastrinemia in the fasted state at the time of the experiment, usually 3 days after surgery (132 ± 36 pmol l^{-1}, $n = 5$, as compared to 15 ± 2 pmol l^{-1}, $n = 16$, in fasted intact rats).

2.4.4. Pylorus ligation

The surgical procedure of acute pylorus ligation in conscious rats has been described in detail previously [40]. In short, each rat (freely fed) was fitted with a microdialysis probe on the dorsal side of the

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