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Stimulatory effect of endogenous orexin A on gastric emptying and acid secretion independent of gastrin

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Abstract

Orexin A (OXA) increases food intake and inhibits fasting small bowel motility in rats. The aim of this study was to examine the effect of exogenous OXA and endogenous OXA on gastric emptying, acid secretion, glucose metabolism and distribution of orexin immunoreactivity in the stomach. Rats equipped with a gastric fistula were subjected to intravenous (IV) infusion of OXA or the selective orexin-1 receptor (OX1R) antagonist SB-334867-A during saline or pentagastrin infusion. Gastric emptying was studied with a liquid non-nutrient or nutrient, using ^{51}Cr as radioactive marker. Gastric retention was measured after a 20-min infusion of OXA or SB-334867-A. Plasma concentrations of OXA, insulin, glucagon, glucose and gastrin were studied. Immunohistochemistry against OXA, OX1R and gastrin in gastric tissue was performed. OXA alone had no effect on either acid secretion or gastric emptying. SB-334867-A inhibited both basal and pentagastrin-induced gastric acid secretion and increased gastric retention of the liquid nutrient, but not PEG 4000. Plasma gastrin levels were unchanged by IV OXA or SB-334867-A. Plasma OXA levels decreased after intake of the nutrient meal and infusion of the OX1R antagonist. Only weak effects were seen on plasma glucose and insulin by OXA. Immunoreactivity to OXA and OX1R were found in the mucosa, myenteric cells bodies and varicose nerve fibers in ganglia and circular muscle of the stomach. In conclusion, endogenous OXA influences gastric emptying of a nutrient liquid and gastric acid secretion independent of gastrin. This indicates a role for endogenous OXA, not only in metabolic homeostasis, but also in the pre-absorptive processing of nutrients in the gut.

Keywords: Gastric acid; Gastric emptying; SB-334867-A; Nitric oxide; Endocrine cells; Neurons; Rat

1. Introduction

The orexins (orexin A (OXA) and orexin B (OXB)) are novel neuropeptides that appear to play a role in appetite and regulation of feeding, arousal, gastrointestinal motility and energy homeostasis (for review, see Ref. [1]). Two orexin receptors have been described, OX1R and OX2R, of which OX1R has a high affinity for OXA, while OX2R has equal affinity for OXA and OXB [2]. The exclusive source of orexins in the central nervous system (CNS) is a small group of neurons in the lateral hypothalamus (LH) [2], a region classically implicated in the control of feeding behavior. The neurons project throughout the CNS with more dense expression of receptors in various nuclei within the hypothalamus (arcuate nucleus (ARC), ventromedial hypothalamus (VMH) and LH), the locus coeruleus (LC) and dorsal motor nucleus of the vagus (DMN) [3,4].

Recent data demonstrate that orexins and orexin receptors are found in the enteric nervous system (ENS) and

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pancreas [5]. Neurons in the submucosal and myenteric plexuses and endocrine cells in the intestinal mucosa and pancreatic islets of the rat and guinea-pig display OXA and orexin receptor immunoreactivity [5,6]. Intravenous (IV) OXA inhibits the fasting migrating motor complex (MMC) [6] and OXA-positive neurons in the gut, like those in the hypothalamus [7], are activated by fasting, indicating a functional role for OXA in the gut in connection with food intake [5]. Intracerebral injection of OXA, but not OXB, increases gastric acid secretion and this effect seems to be dependent on an intact vagal innervation and OX1Rs [8,9]. OXA administered into the DMN or intracisternally facilitates motility of the antrum and relaxation of the fundus of the stomach [10,11]. In addition, intracerebral injection of OXA induces c-*fos* expression in the nucleus of the solitary tract (NTS) and DMN [12], suggesting a role for central orexin in the cephalic phase of acid secretion [8]. A recently developed selective OX1R antagonist (SB-334867-A) has been shown to reduce food intake in *ob/ob*-mice [13]. We have previously demonstrated that the effect of OXA on the MMC is mediated through activation of OX1R and that SB-334867-A [14] shortens the interval between activity fronts of the MMC. This suggests a role for endogenous OXA in the regulation of fasting motility [15].

The aim of this study was to determine if endogenous OXA or peripherally administered OXA influences gastric function by investigating gastric emptying and acid secretion, plasma concentrations of gastrin, and glucose metabolism in rats. In addition, further studies of the distribution of OXA, OXB, OX1R and OX2R as well as gastrin, neuronal nitric oxide synthase (nNOS) and other neuropeptides in the rat stomach were studied with immunohistochemical techniques.

2. Material and methods

2.1. Tissue preparation for immunocytochemistry

Segments (1 cm) of the body and antrum of the stomach were washed and placed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h. After fixation, the preparations were washed and stored in phosphate-buffered saline (PBS) containing sodium azide (1%). Material to be sectioned was cryoprotected overnight (at 4 °C) in PBS containing 30% (w/v) sucrose, embedded in OCT™ (Miles Scientific, Naperville, IL, USA), frozen with liquid N_2, and sectioned (10 μm) using a Leica cryostat-microtome (Leica Microsystems, Deerfield, IL, USA).

For whole mount preparations, segments of the gastric antrum were opened and rectangular sheets of the stomach were stretched, pinned flat on balsa wood and placed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h. The preparations were then washed in PBS and dissected into layers. The whole mount of the myenteric plexus was prepared as described elsewhere [16].

2.2. Immunocytochemistry

The immunocytochemistry methods used in this study have been reported previously [15]. In short the following antibodes were used: OXA-, OX1R- and OX2R-immunoreactivity (rabbit polyclonal, diluted 1:500, Chemicon International, Temecula, CA, USA) using primary antibodies raised in different species in conjunction with species-specific secondary antibodies, coupled to contrasting fluorophores (FITC or Cy3). Primary antibodies were against vasoactive intestinal peptide ((VIP) mouse monoclonal, diluted 1:3000, Center for Ulcer Research/Gastroenteric Biology Center, Antibody/Radioimmunoassay Core, Los Angeles, CA, USA), gastrin (chicken polyclonal, diluted 1:1000, Center for Ulcer Research/Gastroenteric Biology Center), substance P (SP) (rat polyclonal, diluted 1:2000, Accurate, Westbury, NY, USA), nNOS (sheep polyclonal, diluted 1:3000, Chemicon International Inc), and somatostatin (SOM) (rat polyclonal, diluted 1:1000, Pharmigen, San Diego, CA, USA). The preparations were incubated overnight at room temperature. Species-specific antibodies coupled to FITC (diluted 1:500, Jackson ImmunoResearch Labs), coupled to TRITC (diluted 1:500, Kirkegaard and Perry, Gaithersburg, MD, USA) or coupled to Cy3 (diluted 1:2000, Jackson ImmunoResearch Labs).

Parallel control sections that were incubated with normal goat serum instead of primary antibodies were included. No immunostaining was observed when a control IgG was substituted for the primary antibody.

2.3. Surgery

All experiments were performed on male Sprague–Dawley (300–350 g) rats kept under standardized conditions on a commercial diet (Beekay Feeding, ALAB, Sollentuna, Sweden). After an over-night fast, surgery was performed under anesthesia with pentobarbital (50 mg kg^{-1} intraperitoneally; Apoteksbolaget). For gastric acid studies the rats were provided with a plastic gastric fistula placed immediately proximal to the oxyntic gland area near the greater curvature as previously described [17]. Silastic catheters (Dow Corning Co., Midland, MI, USA) were inserted into both jugular veins.

For gastric emptying studies the rats were anesthetized as above. Through a midline incision, an indwelling polyethelene catheter (PE 50, Clay Adams, Becton Dickinson, Parsippany, N J, USA) was implanted into the fundus part of the stomach for administration of the radioactive marker solution. All animals were also supplied with a jugular vein catheter for drug administration. The catheters were tunneled subcutaneously to exit at the back of the animal's neck.

2.4. Studies of gastric acid secretion

Studies of gastric acid secretion began 7–10 days after surgery ($n=7$ for all groups). Food was withheld for 18 h