Inhibitory Effect of Exogenous Orexin A on Gastric Emptying, Plasma Leptin, and the Distribution of Orexin and Orexin Receptors in the Gut and Pancreas in Man

M. Ehrström, T. Gustafsson, A. Finn, A. Kirchgessner, P. Grybäck, H. Jacobsson, P. M. Hellström, and E. Näslund

Division of Surgery (M.E., E.N.), Danderyd Hospital, Karolinska Institutet, Stockholm SE-182 88, Sweden; Departments of Clinical Chemistry (T.G.), Nuclear Medicine (P.G., H.J.), and Gastroenterology and Hepatology (P.M.H., A.F.), Karolinska University Hospital Solna, Karolinska Institutet, SE-171 76 Stockholm, Sweden; and NPS Pharmaceuticals (A.K.), Toronto, Ontario, Canada MSG 1K2

Orexin A (OXA) is a novel peptide that appears to play a role in the regulation of food intake, arousal, and energy balance. The aim of this study was to study the effect of iv infusion of OXA on gastric emptying, appetite, leptin, ghrelin, and glucose metabolism in man (six normal men) and the localization of OXA and orexin receptors (OXRs) 1 and 2 in the human gut. Gastric emptying was studied scintigraphically after ingestion of a 99mTc-labeled omelet and iv infusion of OXA (10 pmol/kg min). Appetite ratings and blood samples were obtained at regular intervals. The immunohistochemical distribution of OXA and OXRIs was examined using antibodies recognizing OXA, OX1R, and OX2R in human gastrointestinal tissue. OXA had no effect on lag phase or gastric half-emptying time. However, the gastric emptying rate was significantly slower without affecting appetite ratings. Plasma concentrations of insulin were increased by OXA, whereas plasma leptin decreased and ghrelin was unchanged. OXA immunoreactivity was observed in a subset of neurons and varicose nerve fibers in the mucosa, ganglia, and circular muscle layer and mucosal endocrine cells in the stomach and small intestine. OXA-immunoreactive cells in the islets of Langerhans contained insulin with a subset expressing OX2R. In conclusion, peripheral OXA seems to slightly affect the regulation of gastric emptying in humans without affecting appetite ratings. OXA decreased plasma levels of leptin, suggesting a possible interaction between leptin and OXA in the regulation of energy homeostasis. (J Clin Endocrinol Metab 90: 2370–2377, 2005)

The orexins [orexin A (OXA) and orexin B] are novel peptides that appear to play a role in the regulation of feeding, arousal and energy homeostasis (for reviews, see Refs. 1 and 2). Initial reports suggested that the orexins exclusively were produced by a small group of neurons in the lateral hypothalamic area (3), a region classically implicated in the control of feeding behavior (4). However, neurons in the submucosal and myenteric plexuses, and endocrine cells in the intestinal mucosa and pancreatic islets, in guinea pig, rat, and humans (5–7) have recently been shown to display OXA and orexin receptor (OXR) immunoreactivity. Several studies have implicated a role for endogenous OXA in the regulation of metabolic homeostasis and preabsorptive processing of nutrients in conjunction with food intake. OXA stimulates motility in the guinea pig isolated colon (5), possibly via pre- and postsynaptic actions in the myenteric plexus (8). Intravenous infusion of OXA inhibits the fasting migrating motor complex (MMC) (6) through activation of the OX1R (9), and orexin-positive neurons in the gut and hypothalamus are activated by fasting (5). In addition, OXA modulates both glucagon and insulin release from the endocrine pancreas, and hypoglycemia stimulates the release of OXA in the rat (10, 11).

It is unclear whether OXA elicits its effects through intrinsic circuits within the enteric nervous system or via the central nervous system (CNS) and the vagus nerve. OXA has been shown to cross the blood-brain barrier by simple diffusion in mice (12). OXA injected intraventricularly or into the dorsal motor complex of the vagus in the rat stimulates pancreatic exocrine secretion, gastric contractility, and acid secretion (13–15), suggesting a vagus nerve-dependent role for OXA in the cephalic phase of digestion. Intraperitoneal infusion of OXA in rat duodenum increases duodenal secretion dependent on previous intake of food (16), and OXA modulates the vagus nerve responses to cholecystokinin (CCK) via CCKA receptors (17), suggesting a role in the regulation of gut-brain signaling. Leptin has inhibitory effects on food intake through central mechanisms (18, 19). Plasma concentrations of OXA are reciprocal to leptin in response to nutritional status in normal-weight humans (20). OX1R immunoreactivity is coexpressed with leptin receptors in feeding regulating neurons in the hypothalamic arcuate nucleus (21) and vagal afferent neurons in rats and humans (17). Thus, the role of OXA in the gut-brain signaling involves endocrine and vagus nerve-
dependent pathways suggested to mediate through leptin-sensitive mechanisms.

The aim of this study was to investigate the effect of an iv infusion of OXA on gastric emptying, appetite, plasma leptin, OXA, glucagon, insulin, and glucose in humans. In addition, the distribution of OXA and OXRs in the human gut and pancreas was studied.

Subjects and Methods

Subjects

Six healthy men were recruited for the study (age, 35.6 ± 2.9 yr old; body mass index, 25.3 ± 1.57 kg/m²). For the immunocytochemical studies, normal tissue from surgical specimens in patient operated on for cancer was used. The Karolinska Institutet North Ethics Committee approved the study. Oral and written informed consent was obtained from all subjects.

Gastric emptying studies

The study was performed in a randomized cross-over fashion on two occasions. The subjects were studied after an overnight fast at 0800 h in the morning, and an indwelling catheter was placed in each antecubital vein. Ten minutes before the intake of a 99mTc-labeled omelet, either saline or OXA [10 pmol/kg/min (Bachem, Bubendorf, Switzerland) dissolved in 0.9%-saline containing 1% albumin (Albumin Kabi, 200 mg/ml; Kabi, Stockholm, Sweden), subjected to sterile filtration, and stored at 20°C until use], was started in one of the iv catheters and continued for 180 min (Bachem, Bubendorf, Switzerland). The samples were centrifuged and decanted. The bound fraction was assessed for 3 min in each occasion. The subjects were studied after an overnight fast at 0800 h, and an indwelling catheter was placed in each antecubital vein. Ten minutes before the intake of a 99mTc-labeled omelet, either saline or OXA [10 pmol/kg/min (Bachem, Bubendorf, Switzerland) dissolved in 0.9%-saline containing 1% albumin (Albumin Kabi, 200 mg/ml; Kabi, Stockholm, Sweden), subjected to sterile filtration, and stored at 20°C until use], was started in one of the iv catheters and continued for 180 min.

The scintigraphic gastric emptying test has previously been described in detail elsewhere (22), and the present technique differs only in that water and not fruit punch was served with the meal. Overnight fasting subjects were studied after ingesting a 1298-kJ omelet with 12–15 MBq 99mTc-labeled macroaggregated albumin (Pulmanon plc; Amersham International, Little Chalfont, UK). Anterior and posterior 1-min acquisitions were performed with the subject in sitting position. Acquisitions were obtained every 5 min during the first 50 min and thereafter every 10 min during 70 min and finally at 180 min. The following parameters were calculated: lag phase, defined as the time period from termination of the meal until 90% radioactivity remained in the stomach; gastric emptying rate, defined as percentage of radioactivity per minute during the linear slope after termination of the lag phase; and half-emptying time (T50), defined as the time for 50% emptying of gastric radioactivity after termination of the meal.

Appetite ratings

Hunger, desire to eat, satiety, and prospective consumption were evaluated 10 min before and 10, 30, 60, 120, and 180 min after the consumption of the omelet using visual analog scales.

Plasma OXA, glucagon, insulin, glucose, leptin, and ghrelin

Blood samples were collected in prechilled heparinized tubes 20 and 10 min before and 10, 20, 30, 40, 50, 60, 90, 120, and 180 min after intake of the omelet. The samples were centrifuged at 4°C for 10 min at 2000 rpm. Plasma was collected and stored at −20°C for analysis of plasma OXA, leptin, glucagon, insulin, and glucose in one series.

OXA levels were determined by RIA, using antisemur RAB-003-30 (Phoenix Europe GmbH, Karlsruhe, Germany). The antisemur, of which each vial was diluted with 13 ml containing PBS, 0.1% Triton X-100, and 0.2% BSA, exhibits 100% cross-reactivity with human, rat, and mouse OXA, and no cross-reactivity with human neuropeptide Y, αMSH, human leptin, human orexin B, human OXA (16–33), and human agouti-related protein fragment (83–132)-NH2. A standard curve of OXA (synthetic OXA, SC 1537, Neosystem, Strasbourg, France; range 7.8–1000 pmol/liter) was prepared by serial dilutions with phosphate buffer (pH 7.4). One hundred microliters of standard solution or crude plasma sample were incubated with 100 μl of antisemur solution at +4°C for 48 h. Thereafter, 100 μl of the tracer, i.e. OXA labeled with 125I (Phoenix Europe) was added and incubated at +4°C for 24 h.

Separation was performed using 500 μl of second antibody, SUSP-3 (Pharmacia Biotech, Uppsala, Sweden) for 30 min at room temperature. After termination of the reaction with 500 μl H2O, the tubes were centrifuged and decanted. The bound fraction was assessed for 3 min in a γ-counter (Wallac, Turku, Finland). All samples were assayed in duplicate in one single run. The performance of the RIA for OXA is shown in Fig. 1. The IC50 was calculated to be 106.4 pmol/liter. The detection limit was 7.8 pmol/liter, and the coefficient of variation was 7% at 62.5 pmol/liter.

Plasma levels of pancreatic glucagon were analyzed with a commercially available RIA kit (Linco Research, St. Charles, MO), which was semiautomated and thereby standardized in the laboratory. A lyophilized ghrelin standard stock at 6 μg/liter (8089-K) was used from which dilutions were performed.

Leptin-like immunoreactivity was analyzed with a commercially available RIA kit (Linco Research), which was semiautomated and thereby standardized in the laboratory. All samples were analyzed in duplicate in a single assay.

Tissue preparation for immunocytochemistry

Tissue from the human gut (corpus and antrum of stomach, duodenum, jejunum, ileum, colon, and pancreas) was obtained from surgical specimens. Immediately after the tissue was removed from the abdomen, it was placed in PBS with 4% formaldehyde (Apoteksbolaget, Umed, Sweden) for 24 h and then transferred to PBS with 1% sodium azide. The tissue was then embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek Europe BV, Zoeterwoude, The Netherlands), and frozen in liquid nitrogen. Cryostat sections (10 μm) were cut and mounted on glass slides and stored at −20°C until immunocytochemistry studies.

Immunocytochemistry

The preparations were exposed to PBS containing 0.5% Triton X-100 (Sigma Chemical Co., St. Louis, MO) and 4% horse serum for 30 min and then incubated with primary antibodies against OXA (affinity-purified rabbit polyclonal, diluted 1:2000; Alpha Diagnostic International, San Antonio, TX), OX1R, and OX2R (affinity-purified, diluted 1:1000; Chemicon International Inc., Temecula, CA). After washing with PBS, the preparations were incubated (3 h) with affinity-purified donkey anti-rabbit secondary antibodies conjugated to indocarbocyanine (Cy3; diluted 1:2000; Jackson ImmunoResearch Laboratories, West Grove, PA), or fluorescein isothiocyanate (FITC; 1:500; Jackson ImmunoResearch Laboratories). Parallel control sections that were incubated with normal goat serum instead of primary antibodies were included. No immuno-
staining was observed when control IgG was substituted for the primary antibody.

Double-label immunocytochemistry was used to identify cells that displayed OXA, OX1R, and OX2R immunoreactivity (rabbit polyclonal, diluted 1:500, Chemicon International) using primary antibodies raised in different species in conjunction with species-specific secondary antibodies, coupled to contrasting fluorophores (FITC or Cy3, as above). Primary antibodies were against vasoactive intestinal polypeptide (VIP) (mouse monoclonal, diluted 1:3000, Center for Ulcer Research/Gastroenteric Biology Center, Antibody/RIA Core, Los Angeles, CA), 5-hydroxytryptamine (5-HT) (mouse monoclonal, diluted 1:5000, Dako Corp., Carpinteria, CA), glucose-dependent insulinotropic polypeptide (rabbit polyclonal, diluted 1:8000, Chemicon International), CCK (rabbit polyclonal, diluted 1:8000, Chemicon International), glucagon-like peptide-1 (GLP-1) (rabbit polyclonal, diluted 1:8000, Chemicon International), CCK (rabbit polyclonal, diluted 1:1000, Chemicon International), insulin (mouse polyclonal, diluted 1:4000, ICN Pharmaceuticals, Costa Mesa, CA), glucagon (guinea pig polyclonal, diluted 1:1000, Linco Research), and somatostatin (SOM; rat polyclonal, diluted 1:1000, Pharmingen, San Diego, CA).

The preparations were incubated overnight at room temperature and then washed with PBS. Species-specific antibodies (coupled to FITC, diluted 1:500, Jackson ImmunoResearch Laboratories, coupled to tetramethylrhodamine isothiocyanate, diluted 1:500, Kirkegaard and Perry, Gaithersburg, MD; or coupled to Cy3, diluted 1:2000, Accurate, Westbury, NY), neuronal nitric oxide synthase (nNOS; sheep polyclonal, diluted 1:500, Jackson ImmunoResearch Laboratories) were added for 3 h, and the slides were then washed in PBS. The tissues were coverslipped in Vectashield (Vector Laboratories, Burlingame, CA). Preparations were examined by using a Radiance 2000 laser-scanning confocal microscope (Bio-Rad Laboratories, San Francisco, CA) attached to an Axioskop 2 microscope (Carl Zeiss Inc., Thornwood, NY). Images of 512 × 512 pixels were obtained and processed by using Photoshop (version 6.0, Adobe Systems, Mountain View, CA).

Statistics and ethics

All data are expressed as mean ± SEM. Results were evaluated with an ANOVA with repeated measures or Wilcoxon signed rank test for matched pairs.

Results

Gastric emptying

Lag phase and T50 were not significantly different during saline and OXA infusion (19.3 ± 4.8 and 16.9 ± 6.2, 65.4 ± 4.2 and 71.7 ± 6.3 min for saline vs. OXA infusion for the lag phase and T50, respectively). However, the gastric emptying rate was significantly slower during OXA infusion (0.73 ± 0.05% per minute), compared with saline (0.91 ± 0.08% per minute, P < 0.05) (Fig. 2).

Appetite ratings

No significant differences were recorded in the visual analog scale ratings of desire to eat, hunger, satiety, or prospective consumption rate during infusion of OXA, compared with saline (Fig. 3).

Plasma OXA, glucagon, insulin, glucose, leptin, and ghrelin

Plasma levels of leptin were numerically higher during OXA infusion. This is likely due to the fact that two of the subjects gained slightly in weight between the saline and OXA infusion days. Plasma levels of leptin decreased significantly during OXA infusion, compared with saline.

Plasma levels of leptin decreased from 4.9 ± 1.3 at time −20 to 4.7 ± 1.2 μg/liter at time 180 min during saline (not significant) and from 7.5 ± 2.8 at time −20 to 5.9 ± 2.1 μg/liter at time 180 min during OXA infusion (P < 0.05) (Fig. 4 and Table 1). No effect of OXA infusion was found on total plasma ghrelin levels (Fig. 4). Plasma OXA was significantly elevated to a peak of 219.0 ± 17.5 pmol/liter at 120 min during iv OXA infusion (P < 0.05) (Fig. 5). Plasma insulin was elevated in the postprandial period (P < 0.05 for time × treatment interaction). No significant differences were found for plasma glucagon and glucose (Fig. 5).

Immunocytochemistry

OXA- and OXR-like immunoreactivity was observed in all regions of the human bowel and islets of Langerhans of the pancreas.

Stomach

OXA-immunoreactive nerve fibers were found in the mucosa of the gastric antrum, and OXA nerve fibers were found to costore SOM and SP. OXA nerve fibers were found in the circular muscle, and a subset of these nerve fibers contained SP (Fig. 6). OXA-immunoreactive endocrine cells were found in abundance in the gastric antrum. These cells coexpressed gastrin but not SOM or 5-HT. Mucosal cells in the gastric corpus expressed OX1R (Fig. 7).

Small intestine

As in the stomach, varicose OXA nerve fibers were found in abundance in the lamina propria of the mucosa and coexpressed SOM and SP. These nerve fibers were found to be in close proximity to SOM-containing enteroeendocrine (EE) cells. OXA-immunoreactive cell bodies were found in the submucosal plexus coexpressing SOM and SP (not illustrated). OXA-immunoreactive cell bodies were also found in the myenteric plexus of the small bowel, a subset of which costored SOM (not illustrated). OXA-positive nerve fibers were found in close proximity to nNOS-immunoreactive neurons in the myenteric plexus (not illustrated). OX1R immunoreactivity was also found in cell bodies in the myenteric plexus. OXA-immunoreactive nerve fibers were found in the
circular muscle layer in the small intestine, and a subset of these was found to costore VIP and nNOS (not illustrated).

OXA-immunoreactive EE cells were found in abundance in the mucosa of the small intestine and in the colon (data for colon not illustrated). The number of OXA-immunoreactive EE cells was most abundant in the duodenum and with decreasing number aborally in the gut. OXA-positive EE cells were found to contain 5-HT and located near SP-immunoreactive nerve fibers (Fig. 8). OXA-positive EE cells did not contain SOM, CCK, GLP-1, or glucose-dependent insulino-tropic polypeptide (not illustrated). Neuronal cell bodies and mucosal epithelial cells displayed OX1R immunoreactivity. In contrast, EE cells expressed OX2R in the small intestine (Fig. 9).

**Pancreas**

OXA-immunoreactive cells were found in islets of Lang-rhans, and a subset of these displayed either insulin or pancreatic polypeptide immunoreactivity (Fig. 10). OXA-containing islet cells did not contain SOM or glucagon (not illustrated).

**Fig. 3.** Mean ± SEM appetite ratings after a solid meal (310 kcal) during saline and iv OXA infusion (10 pmol/kg·min) (NS for all ratings) in six healthy men.

**Fig. 4.** Mean ± SEM plasma (P) leptin and ghrelin concentrations after a solid meal during saline and iv OXA infusion (10 pmol/kg·min) in six healthy men. The infusion was started at −10 min and the meal consumed at 0 min (P < 0.05 for change between −20 and 180 min for leptin, NS for ghrelin).
Discussion

This study demonstrates that peripheral OXA significantly decreases the rate of gastric emptying in humans. Plasma leptin levels are decreased, whereas plasma insulin levels are increased by OXA. Appetite ratings and plasma levels of glucagon and glucose were unaffected by OXA. OXA and OXR-like immunoreactivity are found throughout the human gut. The physiological implication and mechanism of action of our finding that OXA decreases the rate of gastric emptying may reflect a predominant inhibitory tone on gastric emptying during fasting when gastric acid is accumulated in preparation for the next digestive process. Plasma levels of OXA are increased during fasting in humans (21); thus, the likely pharmacological elevated plasma levels of OXA after food intake achieved in this study reflects a truly artificial condition that would have its greatest impact on appetite ratings. However, because appetite ratings were not affected, it can be speculated that peripheral OXA constitutes its own pool with no communication with central OXA pathways.

We previously demonstrated that peripheral OXA inhibits the migrating motor complex in rats (6), and thus, OXA might have a general inhibitory effect on gut motility during prolonged fasting. In our previous study on fasting motility in the rat, we found no effect of vagotomy on the effect of OXA on fasting motility, suggesting a peripheral effect of the peptide (9). OXA injected intraventricularly or into the dorsal motor complex of the vagus nerve in rat stimulates pancreatic exocrine secretion, gastric contractility, and acid secretion (13–15). Again, central and peripheral OXA may serve different effects on gut motility. It has been reported that OXA can pass the blood-brain barrier (12), yet the peptide was modified by attaching iodine, which may influence the ability of OXA to pass into the brain. Further support of a local effect of OXA in the gut is that intraarterial infusion of OXA decreases duodenal secretions (16). The morphological data presented in this study further support the idea that peripheral OXA acts within the enteric nervous system because OX1Rs are found in the myenteric plexus of the intestine.

In contrast to our initial hypothesis, we found no effect of peripherally administered OXA on appetite ratings. Cen-

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<th>Subject</th>
<th>Control Difference 180 to −20 min</th>
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<td>5</td>
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Fig. 5. Mean ± SEM plasma (P) insulin, glucose, glucagon, and OXA concentrations after a solid meal during saline and iv OXA infusion (10 pmol/kg/min) in six healthy men. The infusion was started at −10 min and the meal consumed at 0 min (insulin (P < 0.001 for time, NS for treatment, P < 0.05 for time × treatment), glucose (P < 0.001 for time, NS for treatment and time × treatment), glucagon (P < 0.001 for time, NS for treatment and time × treatment), and OXA (P < 0.001 for time, treatment, and time × treatment).
trally administered OXA stimulates food intake in rodents (3). This lends further support to the idea that a peripheral and central pool of OXA exists and that peripheral OXA under physiological conditions does not cross the blood-brain barrier. Interestingly, exogenous OXA results in decreased plasma leptin levels. The significance of this is not clear. Prolonged fasting increases the plasma concentrations of OXA in rat (10) and man (23), whereas plasma levels of leptin fall during fasting (20). This suggests that there might exist a gut-adipocyte interaction in which high plasma OXA signals starvation and may stimulate food intake by decreasing plasma leptin levels and thus interacting with leptin sensitive central mechanisms. Conversely, high plasma triglyceride concentrations and high-fat diets increase OXA gene expression centrally (24, 25). In fasting rats, opposite data have been reported in which plasma levels of leptin increased 60 and 120 min after sc administration of OXA (26). However, the reported data may be confounded by a mechanically induced release of leptin from sc adipose tissue after injection of OXA. With regard to ghrelin, we found no effect of OXA on the plasma levels of that peptide, suggesting separate mechanisms for their orexigenic effects.

In concordance with previous studies in rats (10), OXA-like immunoreactivity was found to be colocalized with insulin in the human pancreas, suggesting that OXA may have an effect on glucose metabolism in humans. Indeed, we found an effect of OXA on the time course of insulin secretion with elevated plasma insulin in the postprandial period. Again, the physiological implication of this is not clear because plasma OXA is elevated in the fasted state. Previous studies in rats on the effect of OXA on insulin and glucose show conflicting results. Both increased (11) and decreased (10) plasma levels of insulin have been reported in fasting rats, thus making our observation of increased plasma insulin postprandially point in the direction of an incretin action of OXA. Such properties must be disclosed in further metabolic studies.

The finding of OXA and OXRs in the duodenum of human mucosa of the gastric antrum (arrow, A). OXA-nerve fibers contain SOM (arrow, B) and SP (not illustrated). OXA-immunoreactive nerve fibers are found in the circular muscle (arrow, C), and a subset contains SP (arrow, D). Horizontal markers, 10 μm.

Fig. 6. OXA-immunoreactive nerve fibers are abundant in the mucosa of the gastric antrum (arrow, A). OXA-nerve fibers contain SOM (arrow, B) and SP (not illustrated). OXA-immunoreactive nerve fibers are found in the circular muscle (arrow, C), and a subset contains SP (arrow, D). Horizontal markers, 10 μm.

Fig. 8. OXA-immunoreactive EE cells are abundant in the mucosa of the small intestine (A; arrow) as are OXA-immunoreactive nerve fibers in the lamina propria (A; arrowhead). OXA-immunoreactive EE cells are in close approximation with SP-immunoreactive nerve fibers in the lamina propria (B; arrow). EE cells in the mucosa costore OXA (C) and 5-HT (D) but not SOM, CCK, or GLP-1 (not illustrated). Horizontal markers, 10 μm.

Fig. 7. OXA-immunoreactive endocrine cells are abundant in the gastric antrum (A–C). These cells do not contain SOM (A) or 5-HT (B); however, they contain gastrin (C). OX1R is expressed by cells in the mucosa (arrow, D) in the gastric corpus; therefore, OXA is likely to modulate gastric acid secretion. Horizontal markers, 10 μm.

Fig. 9. OX1R immunoreactivity is displayed by neuronal cell bodies (A) and mucosal epithelial cells (B). OX2R is expressed by endocrine cells (C) in the duodenum. Horizontal markers, 10 μm.
mans confirms and extends our previous results demonstrating OXA and OXR in the gut of guinea pigs and rats (5). One previous study regarding the localization of OXA in the human gut has been published. Yet this study did not include the immunoreactivity of OXRs and did not use double staining against other peptides (7). OXA-like immunoreactivity was found both in nerve fibers in the lamina propria of the mucosa and in the circular muscle layer in humans. The fibers also exhibited immunoreactivity to VIP in both locations and nNOS in the circular muscle, which supports previous findings (5, 9). VIP submucosal neurons project to myenteric neurons, arterioles, and mucosa, and in conjunction with nitric oxide and ATP (27, 28), VIP seems to play an important role in the mediation of the nonadrenergic noncholinergic relaxation of the gut during peristalsis. OXA also seems to affect gut motility because peripheral administration disrupts (6) and the antagonist SB-334867-A shortens the intervals of the MMC (9). The effect of OXA on the MMC seems to at least partly involve a nitric oxide-dependent pathway because the effect of OXA on the MMC was blocked by the administration of an nNOS inhibitor (9). Because OXA, VIP, and NOS are colocalized in nerve fibers of the mucosa and circular muscle, it is likely that these structures are innervated by orexin neurons, thereby providing possible pathways by which orexin can modulate gastrointestinal secretomotor function.

In the mucosa of the human duodenum, a subset of the OXA-immunoreactive neurons was found to coexpress 5-HT-like immunoreactivity. 5-HT is released in response to mucosal stimulation and activates both intrinsic (29, 30) and extrinsic (31) primary afferent neurons, resulting in reflex alteration of gut function. 5-HT has been suggested to participate in the initiation of the peristaltic reflex, and OXA seems to enhance the peristaltic reflex in vitro (5). Our finding of the presence of the OX2R on cells that contain 5-HT suggests that OXA may modulate 5-HT release and that this may be one mechanism for OXA to influence gut motility.

This study demonstrates that peripheral OXA slightly slows the gastric emptying rate without affecting appetite ratings. In addition, OXA decreases the plasma levels of leptin over time. OXA and OXRs are present throughout the human gut and pancreas. Based on the localization of OXA and OXRs and effects on gastric emptying rate without affecting appetite ratings, it seems likely that endogenous OXA may have a role in modulating food intake and gastrointestinal function in the preabsorptive processing of nutrients. It seems as though peripheral OXA decreases plasma leptin, suggesting that peripheral OXA may modulate long-term energy balance through leptin pathways. Even if we cannot disclose the exact point of action of OXA today, continuous work ahead by the use of selective OX1R or OX2R antagonists with and without CNS penetration to the CNS may help in our understanding of the role of the orexins in the gut.

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Address all correspondence and requests for reprints to: Marcus Ehrström, M.D., Department of Surgery, Danderyd Hospital, SE-182 88 Stockholm, Sweden. E-mail: marcus.ehrstrom@kir.ds.sll.se.

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