Short fasting dramatically decreases rat duodenal secretory responsiveness to orexin A but not to VIP or melatonin

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Flemström, Gunnar, Markus Sjöblom, Gunilla Jedstedt, and Karl E. O. Åkerman. Short fasting dramatically decreases rat duodenal secretory responsiveness to orexin A but not to VIP or melatonin. Am J Physiol Gastrointest Liver Physiol 285: G1091–G1096, 2003. First published July 17, 2003; 10.1152/ajpgi.00193.2003.—Orexins are involved in the central nervous control of appetite and behavior, and in addition, they are present in endocrine cells and/or neurons in the intestine. The role of these peptides in peripheral regulation of intestinal secretion has not been investigated. We thus compared the effects of orexin A and some established secretagogues on duodenal HCO₃⁻ secretion in fed rats with effects in rats exposed to short (overnight) food deprivation. Rats were anesthetized with thiobarbiturate, a 12-mm segment of proximal duodenum with intact blood supply was cannulated in situ, and the alkaline secretion was titrated by pH stat. Secretagogues were supplied specifically to the duodenum by close intra-arterial infusion. Orexin A (60–600 pmol·kg⁻¹·h⁻¹) caused marked and dose-dependent stimulation of the duodenal secretion in fed animals but did not affect secretion in overnight food-deprived animals. Similarly, short fasting caused a 100-fold increase in the amount of the muscarinic agonist bethanechol (from 50 to 5,000 nmol·kg⁻¹·h⁻¹) required for stimulation of the secretion. In contrast, the secretory responses to VIP (50–1,000 pmol·kg⁻¹·h⁻¹) and melatonin (20–200 nmol·kg⁻¹·h⁻¹) were not affected. The appetite-regulating peptide orexin A is thus a stimulant of intestinal secretion, but the response to this peptide as well as the muscarinic agonist bethanechol is markedly dependent on previous intake of food. Overnight fasting is a standard experimental procedure in studies of gastrointestinal physiology and pathophysiology in humans and in intact animals have been conducted after overnight fast. Because the presence of food itself has a considerable effect on intestinal function (14), we have compared the actions of orexins and other intestinal secretagogues in sedentary animals and in animals deprived of food for a short period. The duodenal mucosal HCO₃⁻ secretion, chosen for study, is an interesting model of intestinal anion transport. This secretion is controlled through central and peripheral neuroendocrine mechanisms. It is a main mechanism of duodenal mucosal defense against acid discharged from the stomach (11) and is deficient in patients with chronic and acute duodenal ulcer disease (16, 19, 29).

Melatonin influences a variety of biological processes, including behavior, and enkephalin and enkephalin-containing cells in the gastrointestinal tract have been identified to be a major source of melatonin production (5). The total amount of melatonin present in the intestine is thus ~400× larger than in the central nervous system (5, 17, 34). Recent studies from our group have shown that melatonin, acting at MT₂ receptors, stimulates mucosal HCO₃⁻ secretion in rat duodenum in vivo (41, 42) and, in addition, mediates centrally elicited neural stimulation of the alkaline secretion (42). Melatonin also induces intracellular calcium ([Ca²⁺]i) signaling in isolated human and rat duodenal enterocytes, strongly suggesting an action at enterocyte membrane receptors (43).

Cholinergic transmission is of main importance in central as well as peripheral nervous control of gastrointestinal function. Stimuli mediated by the vagal nerves or parenteral administration of cholinergic agonists, including the predominantly muscarinergic agonist bethanechol, increase the duodenal HCO₃⁻ secretion in all species tested, including humans (1, 10, 15, 37). Use of subtype-selective ligands demonstrated that carbachol-induced [Ca²⁺]i signaling in enterocytes isolated from human and rat duodenum is mediated by muscarinic M₃ receptors (8). VIP is a potent stimulant of intestinal secretion, and infusion of VIP increases in peripheral regulation of intestinal function has, however, not been investigated in detail. Orexin cells in the hypothalamus are stimulated by fasting and inhibited by feeding (6). By tradition (32), experimental studies of gastrointestinal physiology and pathophysiology in humans and in intact animals have been conducted after overnight fast. Because the presence of food itself has a considerable effect on intestinal function (14), we have compared the actions of orexins and other intestinal secretagogues in sedentary animals and in animals deprived of food for a short period. The duodenal mucosal HCO₃⁻ secretion, chosen for study, is an interesting model of intestinal anion transport. This secretion is controlled through central and peripheral neuroendocrine mechanisms. It is a main mechanism of duodenal mucosal defense against acid discharged from the stomach (11) and is deficient in patients with chronic and acute duodenal ulcer disease (16, 19, 29).

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OREXINS WERE FIRST ISOLATED as hypothalamic appetite-increasing peptides (9, 38) and appear to be involved in short-term regulation of feeding rather than long-term regulation of body weight (6, 46). Effects are mediated via two G protein-coupled receptors denoted OX₁ and OX₂ (21, 24). Orexins and receptors were also recently discovered in numerous endocrine (enterochromaffin) cells in the gastrointestinal tract and are present in the enteric nerves (21, 24, 27). The role of these compounds in peripheral regulation of intestinal function has, however, not been investigated in detail. Orexin cells in the hypothalamus are stimulated by fasting and inhibited by feeding (6). By tradition (32), experimental studies of gastrointestinal physiology and pathophysiology in humans and in intact animals have been conducted after overnight fast. Because the presence of food itself has a considerable effect on intestinal function (14), we have compared the actions of orexins and other intestinal secretagogues in sedentary animals and in animals deprived of food for a short period. The duodenal mucosal HCO₃⁻ secretion, chosen for study, is an interesting model of intestinal anion transport. This secretion is controlled through central and peripheral neuroendocrine mechanisms. It is a main mechanism of duodenal mucosal defense against acid discharged from the stomach (11) and is deficient in patients with chronic and acute duodenal ulcer disease (16, 19, 29).

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the HCO$_3^-$ transport by the duodenal mucosa in all species tested (10, 13, 47). The peptide does not affect [Ca$^{2+}$], signaling in human enterocytes in primary culture (8) but causes a marked increase cAMP production in rat and guinea pig isolated duodenal crypt and villus enterocytes (35, 36).

We demonstrate in the present study that overnight fasting abolishes the duodenal secretory response to the appetite-regulating peptide orexin A and profoundly downregulates that to the muscarinergic agonist bethanechol. In contrast, responses to VIP and melatonin are little affected.

**MATERIALS AND METHODS**

*Chemicals and drugs.* Orexin A (human, rat, mouse) was purchased from Peninsula (Merseyside, UK), and bethanechol (carbamyl-$\beta$-methylcholine chloride), VIP (human, rat, porcine), and BSA were from Sigma (St. Louis, MO). Melatonin and the anesthetic 5-ethyl-5-[(1-methyl-propyl)-2-thio-barbiturate (Inactin) were from Research Biochemicals International (Natick, MA). Bethanechol, melatonin, and the anesthetic were stored in powder form and dissolved in isotonic saline at pH 7.4 on the day of use. Orexin A and VIP were stored at $-20^\circ$C in stock solutions also containing 0.5 mg/ml BSA.

*Animal preparation and measurements.* All experiments were approved by the Uppsala Ethics Committee for Experiments with Animals. Male F$_1$-hybrids of Lewis $\times$ Dark Agouti rats (Animal Department, Biomedical Center, Uppsala, Sweden), weighing 220–260 g were placed in a conditioning unit under standardized temperature and light conditions (21–22°C, 12:12-h light-dark cycle). The rats were kept in cages in groups of two or more and had, unless deprived of food, free access to pelleted food (Ewos, Söderfältje, Sweden).

The animals were deprived of food (fasted) for 16 h (overnight) before experiments but had free access to drinking water. Fed rats from the same batch had continuous access to their regular supply of pelleted food and water. Experiments were started by anesthetizing the animal at close to 9 AM with Inactin (120 mg/kg body wt ip). Anesthesia was performed within the Animal Department by the person who had previously handled the animals. Subsequently, the rats were tracheotomized with a tracheal tube to facilitate respiration, and body temperature was maintained at 37–38°C throughout experiments by a heating pad controlled by a rectal thermistor probe. The surgical and experimental procedures have been described previously (12, 42). A brief summary is given, and some modifications are described here. A femoral artery and vein were catheterized with PE-50 polyethylene catheters (Becton-Dickinson, Parsippany, NJ).

For continuous recordings of systemic arterial blood pressure, the arterial catheter containing 20 IU/ml heparin isotonic saline was connected to a transducer operating a PowerLab system (AD Instruments, Hastings, UK). The vein was used for infusion of Ringer solution [in (mM) 145 Na$^+$, 124 Cl$^-$, 2.5 K$^+$, 0.75 Ca$^{2+}$, and 25 HCO$_3^-$] at a rate of 1.0 ml/h. The latter was made to compensate for fluid loss and to avoid acid-base changes during the experiments. Blood acid-base balance was controlled (AVL, Compact 3, Graz, Austria) in 40-$\mu$l arterial blood samples taken at the start and end of experiments. Values of blood pH were between 7.30 and 7.45, and changes in base excess were within $\pm$ 3 mM.

The abdomen was opened by a midline incision, and the gastric pylorus was ligated with a suture. To avoid bile and pancreatic secretion entering the intestine, the common bile duct was always catheterized close to its entrance to the duodenum with a PE-10 polyethylene tubing (Becton-Dickinson). For measurement of duodenal mucosal HCO$_3^-$ secretion, a 12-mm segment of duodenum with its blood supply intact, starting 10–12 mm distal to the pylorus and thus devoid of Brunner’s glands, was cannulated in situ between two glass tubes connected to a reservoir. Fluid (10 ml of 154 mM NaCl), maintained at 37°C by a water jacket, was rapidly circulated by a gas lift of 100% oxygen. HCO$_3^-$ into the luminal perfusate was continuously titrated with 50 mM HCl at pH 7.4 under automatic control of a pH stat system (Radiometer, Copenhagen, Denmark). After completion of the operative setup, the abdomen was closed with sutures and the animal was left undisturbed for 1 h for stabilization of cardiovascular, respiratory, and gastrointestinal functions.

*Intra-arterial infusion to the duodenum.* All secretagogues were administered to the duodenum by close intra-arterial infusion, described previously by Sjöblom et al. (42). Only small amounts of the compounds were thus required, minimizing any central nervous action. The hepatic artery was cannulated, tied 3–4 mm proximal to its entrance into the liver, and perfused in the retrograde direction at 17 $\mu$l/min (Fig. 1). This perfusion results in distribution of the perfusate mainly to the duodenum (via the cranial pancreatico-duodenal artery) and pancreas. The distribution was checked visually at the start of experiments by intra-arterial injection of a small amount (~0.1 ml) of a marker dye (Evans blue, 2% solution in saline).

*Data analyses.* Descriptive statistics are expressed as means $\pm$ SE. Rates of alkaline secretion by the duodenum are expressed as microequivalents of base (HCO$_3^-$) per centimeter of intestine per hour. The secretion and the mean
affected neither the alkaline secretion nor the mean rates in all groups tested and infusion of vehicle alone contents within the duodenal lumen. The examined the appearance of the proximal part of the small intes-

animals not deprived of their regular food. Infusion of orexin A did not affect the mean arterial blood pressure in either group (not shown).

Decreased sensitivity to bethanechol. The cholinomimetic agonist bethanechol acts predominantly at muscarinic receptors. Close intra-arterial infusion of bethanechol (50, 500, and 5,000 nmol·kg⁻¹·h⁻¹) to the duodenum in fed animals caused dose-dependent increases in mucosal HCO₃⁻ secretion (from 13.7 ± 1.71 to 28.3 ± 5.97 μeq·cm⁻¹·h⁻¹). Already the lowest dose infused (50 nmol·kg⁻¹·h⁻¹) significantly (P < 0.05) increased the alkaline secretion (Fig. 3).

In overnight food-deprived animals, the highest dose tested (5,000 nmol·kg⁻¹·h⁻¹) was required for stimulation (P < 0.05) of the secretion (from 13.1 ± 1.42 to 21.4 ± 4.11 μeq·cm⁻¹·h⁻¹). Overnight fasting thus induced an ~100-fold decrease in sensitivity to bethanechol. The present (close intra-arterial infusions) of bethanechol did not significantly affect the mean arterial blood pressure (not shown).

Infusion of VIP and melatonin. VIP is a potent stim-

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orexin A and duodenal secretion

Fig. 2. Orexin A stimulates the HCO₃⁻ secretion by the duodenal mucosa in continuously fed but not in overnight-fasted animals. Compounds were administered by close intra-arterial infusion, and the secretion was continuously titrated (pH stat). Means ± SE of HCO₃⁻ secretion are shown (n = 6 in fed and n = 7 in fasted animals). No changes in mean arterial blood pressure were observed in these experiments (not shown).

RESULTS

Food deprivation abolishes stimulation by orexin A. The stomach in overnight food-deprived animals had a smaller volume than that in animals with continuous access to food, but there were no visual differences in the appearance of the proximal part of the small intestine. In all groups, there were only small amounts of contents within the duodenal lumen. The examined segment spontaneously secreted HCO₃⁻ at steady basal rates in all groups tested and infusion of vehicle alone affected neither the alkaline secretion nor the mean arterial blood pressure.

Close intra-arterial infusion of orexin A (60, 240, and 600 pmol·kg⁻¹·h⁻¹) to the duodenum did not affect (P > 0.05) the alkaline secretion in overnight fasted animals (Fig. 2). In contrast, there was a significant (P < 0.05) and dose-dependent increase in secretion (from 10.6 ± 2.48 to 19.9 ± 3.64 μeq·cm⁻¹·h⁻¹) in
 Orexin A caused robust stimulation of the HCO₃⁻ secretion in fed animals but did not affect secretion in overnight fasted animals. Orexins (A and B) were originally discovered in the central nervous system as peptides that increase the appetite of fed animals (38). Subsequently, both orexins were found also in neurons and in neuroendocrine cells of the intestine (21, 22), and orexin immunoreactivity is colocalized with VIP and choline acetyltransferase (30). OX₁ as well as OX₂ receptors are expressed in the intestine, although in different cell types (30). OX₁ receptors are expressed mainly in neurons, whereas endocrine cells express OX₂ receptors. The roles played by orexins in the gastrointestinal tract are not well understood. The peptides have been reported both to increase (21) and to reduce (39) motility in the small intestine. In the colon, orexins induce stimulation of motility (6, 22). Orexins thus probably act at several levels, and some of their different actions are very likely mediated via other neurohumoral systems in the intestine. Nitric oxide-linked mechanisms are proposed to mediate orexin-induced inhibition of motility (39). On the other hand, contraction of isolated ileum seems to occur through release of acetylcholine (28). Fasting abolished the HCO₃⁻ secretory response to orexin A and markedly reduced the sensitivity to bethanechol (Figs. 2 and 3).

If secretion stimulated by orexins (in fed animals) is controlled via cholinergic mechanisms similar to those for contraction, reduction in cholinergic responsiveness by fasting could induce a secondary attenuation of responses to orexins. Muscarinic stimulation (carbachol) has been shown to directly activate intracellular Ca²⁺ mobilization in cultured (8) and actutely isolated (43) duodenal enterocytes.

The mechanisms by which feeding promotes responses to orexin and bethanecol are far from clear. Stimulation of secretion might require or be potentiated by release of endorphin from the pituitary gland. In the intestine, although in different cell types (30), OX₁ as well as OX₂ receptors are expressed in the intestine, although in different cell types (30). OX₂ receptors are expressed mainly in neurons, whereas endocrine cells express OX₂ receptors. The roles played by orexins in the gastrointestinal tract are not well understood. The peptides have been reported both to increase (21) and to reduce (39) motility in the small intestine. In the colon, orexins induce stimulation of motility (6, 22). Orexins thus probably act at several levels, and some of their different actions are very likely mediated via other neurohumoral systems in the intestine. Nitric oxide-linked mechanisms are proposed to mediate orexin-induced inhibition of motility (39). On the other hand, contraction of isolated ileum seems to occur through release of acetylcholine (28). Fasting abolished the HCO₃⁻ secretory response to orexin A and markedly reduced the sensitivity to bethanechol (Figs. 2 and 3).

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**DISCUSSION**

We demonstrate here that feeding induces, or very markedly potentiates, the response of the duodenal HCO₃⁻ secreting epithelium to some stimuli but not to others. The HCO₃⁻ secretion is under central as well as peripheral neurohumoral influence, and intracerebroventricular administration of some neuropeptides and the α₁-selective adrenoceptor agonist phenylephrine stimulates the duodenal secretion in the rat (10, 12, 25, 42). The centrally elicited secretory responses are mediated by vagal and sympathetic neural pathways and by release of β-endorphin from the pituitary gland. In the present study, all secretagogues were administered to the duodenum by close intra-arterial infusion (Fig. 1). Only very small amounts were thus required for stimulation of secretion, strongly suggesting that rises in secretion reflected stimulation of the duodenal mucosa per se and that central nervous actions did not occur. The absence of a depression of the mean arterial blood pressure after infusion of VIP or bethanechol provided further evidence that effects of intra-arterially administered compounds were confined to the duodenum in the present study.

**Fig. 4.** VIP is a potent stimulant of duodenal mucosal HCO₃⁻ secretion in vivo. Means ± SE of HCO₃⁻ secretion are shown, and there were no significant differences between fed and fasted animals (n = 6 in the fed and n = 8 in the fasted group). The close intra-arterial infusion of VIP did not affect the mean arterial blood pressure (not shown).

**Fig. 5.** The neurohormone melatonin is a stimulant of duodenal secretion. No significant changes between the fed (n = 6) and fasted (n = 7) were found, and melatonin did not affect the mean arterial blood pressure.
ated by food-induced intestinal motility (14). An attractive alternative explanation for the changes in sensitivity to orexin A and the muscarinic agonist would be that food constituents either directly or indirectly by central or peripheral mechanisms stimulate the activity or expression of signal pathways or receptors in the intestinal mucosa. Unfortunately, the information on the role of feeding on intestinal function is limited. Fasting and glucopenia stimulate orexin production in the lateral hypothalamus (reviewed in Ref. 7). Fasting also increases orexin receptor expression in this region (26). Lowered glucose levels stimulate orexin release in the endocrine pancreas (31), and hypoglycemia increases plasma orexin levels (23). Thus there seems to be a close link between the glucose-sensing system and orexin release (reviewed in Ref. 7). Our study indicates that there exists additional links between the feeding status and the orexin system. The feeding status thus not only regulates the synthesis and release of orexins but can also control the responsiveness to the peptides on intestinal cells. Alternatively, fasting may inhibit orexin and or muscarinic responses by receptor desensitization.

In contrast to the findings with orexin A and melatonin, the HCO$_3^-$ secretory responses to VIP and melatonin were not affected by overnight fasting. This demonstrates that the feeding does not cause a general increase in responsiveness of secretory peptides but has a more selective action. One explanation for the difference seen here could be different signal pathways used for stimulation of HCO$_3^-$ transport. Orexins and muscarinic M$_3$ agonists couple to intracellular Ca$^{2+}$ mobilization and phospholipase C activation (8, 24). VIP is known to stimulate the secretion via cAMP-dependent mechanisms (35, 36), whereas the signal pathway used by melatonin may be different. Melatonin receptors are usually coupled to inhibitory G$_i/o$ proteins (3, 45). However, melatonin induces cytosolic Ca$^{2+}$ mobilization in primary cultures of ovine pars tuberalis and HEK 293 cells stably expressing MT$_1$ receptors (4), and a recent study demonstrated that melatonin, acting at MT$_2$ receptors, mobilizes intracellular Ca$^{2+}$ in isolated human and rat duodenal enterocytes (43).

Knowledge about the HCO$_3^-$ transport processes in duodenal mucosa originates from numerous studies involving amphibian mucosa in vitro, isolated mammalian duodenal enterocytes and cell membranes (10), and, more recently, from studies of genetically modified mice. Anion transport processes in duodenal epithelium are, in general, similar to those of more distal small intestine (2). An amiloride-sensitive Na$^+/H^+$ exchanger extrudes acid (18, 33). Duodenal enterocytes import HCO$_3^-$ at the basolateral membrane by Na$^+/HCO_3^-$ cotransport and export HCO$_3^-$ by Cl$^-$/HCO$_3^-$ exchange as well as via an apical anion conductive pathway (18, 20, 44). The CFTR is the membrane-spanning conductance transporting HCO$_3^-$ as well as Cl$^-$ into the duodenal lumen (40). Interactions between the CFTR channel and the major apical anion exchanger downregulated in adenoma (20), as well as the Cl$^-$ to HCO$_3^-$ ratio of the CFTR channel, may relate to the high rates of alkali secretion by the duodenal epithelium.

Bicarbonate and mucus form a “mucus-bicarbonate barrier” on top of the duodenal mucosa and are considered a major line of defense against acid-induced mucosal injury (11). The present study demonstrates that orexin A is a stimulant of the duodenal secretion and that short fasting abolishes the responsiveness to orexin and markedly reduces that to bethanecoch. Overnight food deprivation is, by tradition (32), a standard experimental procedure in studies of gastrointestinal function and pathophysiology in humans and animals. The present observations strongly suggest that the many studies made on neuroendocrine control of intestinal secretion and effects of drug therapy may require reevaluation with respect to feeding status.

DISCLOSURES

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