Localization and effects of orexin on fasting motility in the rat duodenum

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Received 27 May 2001; accepted in final form 15 October 2001

Näslund, E., M. Ehrström, J. Ma, P. M. Hellström, and A. L. Kirchgessner. Localization and effects of orexin on fasting motility in the rat duodenum. Am J Physiol Gastrointest Liver Physiol 282: G470–G479, 2002; 10.1152/ajpgi.00219.2001.—The orexins [orexin A (OXA) and orexin B (OXB)] are novel neuropeptides that increase food intake in rodents. The aim of this study was to determine the distribution of orexin and orexin receptors (OX1R and OX2R) in the rat duodenum and examine the effects of intravenous orexin on fasting gut motility. OXA-like immunoreactivity was found in varicose nerve fibers in myenteric and submucosal ganglia, the circular muscle, the mucosa, submucosal and myenteric neurons, and numerous endocrine cells of the mucosa. OXA neurons displayed choline acetyltransferase immunoreactivity, and a subset contained vasoactive intestinal peptide. OXA-containing endocrine cells were identified as enterochromaffin (EC) cells based on the presence of 5-hydroxytryptamine immunoreactivity. OX1R was expressed by neural elements of the gut, and EC cells expressed OX2R. OXA at 100 and 500 pmol·kg−1·min−1 significantly increased the myoelectric motor complex (MMC) cycle length compared with saline. Similarly, OXB increased the MMC cycle length at 100 pmol·kg−1·min−1, but there was no further effect at 500 pmol·kg−1·min−1. We postulate that orexins may affect the MMC through actions on enteric neurotransmission after being released from EC cells and/or enteric neurons.

In the central nervous system, orexins are produced exclusively by a small group of neurons in the lateral hypothalamic area (5, 8, 10, 21, 24), a region classically implicated in the control of feeding (23). Orexin mRNA expression is increased by fasting in rodents (19, 24), and OXA increases food intake (presumably via OX1R) when injected into the third ventricle or lateral hypothalamus (6, 24, 25, 30). In addition, a selective OX1R antagonist reduces feeding over several days as well as feeding stimulated by fasting or administration of OXA (1). Furthermore, OXA increases gastric acid secretion, however, only when given centrally and with an intact vagus nerve (26), suggesting a role for orexins in the brain-gut axis.

Orexin and orexin receptors are also found in the enteric nervous system (ENS) and pancreas (14). OXA and orexin receptor immunoreactivity is displayed by a subset of neurons in the submucosal and myenteric plexus and endocrine cells in the mucosa of the ileum and pancreatic islets. OXA stimulates motility in the guinea pig isolated colon (14) and insulin release from perfused rat pancreas (20). Moreover, the orexin-positive neurons in the gut, like those in the hypothalamus, are activated by fasting, indicating a functional response to food status in these cells (14).

The aim of this study was to determine the distribution of orexin and orexin receptors in the rat duodenum and examine the effects of orexins on fasting motility in the proximal small intestine.

MATERIAL AND METHODS

Tissue preparation. Male Sprague-Dawley rats (300–350 g; n = 25) were killed with an intravenous (IV) injection of pentobarbital sodium (Apoteksbolaget, Umeå, Sweden). The local ethics committee for animal experimentation in northern Stockholm, Sweden approved the experimental protocol. Segments (1 cm) of duodenum (just distal to the pylorus) were removed, washed through the lumen, and placed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h. After the preparations were fixed, they were washed and stored in PBS containing sodium azide (1%). Material to be

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sectioned was cryoprotected overnight (at 4°C) in PBS containing 30% (wt/vol) sucrose, embedded in optimum cutting temperature compound (Miles Scientific, Naperville, IL), frozen with liquid N₂, and sectioned (10 μm) by using a Leica cryostat-microtome (Leica Microsystems, Deerfield, IL).

For whole mount preparations, segments of duodenum were opened along the mesenteric border and the resulting rectangular sheet of intestine was stretched, pinned flat on balsa wood, and fixed as above. After the preparations were fixated, they were washed in PBS and then dissected into layers. Two whole mounts, one containing the submucosal and the other the myenteric plexus, were prepared as previously described (13).

Immunocytochemistry. For immunocytochemistry, preparations of gut were exposed to PBS containing 0.5–1.0% Triton X-100 and 4% horse serum for 30 min. The preparations were then exposed for 24–48 h at 4°C to antibodies raised against OXA or orexin receptors (see Table 1). After the preparations were washed with PBS, they incubated (for 3 h) with affinity-purified species-specific secondary antibodies conjugated to tetramethylrhodamine isothiocyanate (diluted 1:500; Kirkegaard and Perry, Gaithersburg, MD), indocarbocyanine (Cy-3; diluted 1:2,000, Jackson ImmunoResearch Laboratories, West Grove, PA), or FITC (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA), or FITC (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA). The tissues were placed on coverslips in Vectashield (Vector Laboratories, Burlingame, CA). In every experiment, parallel control sections were included that were incubated with normal goat serum instead of primary antibodies.

Double-label immunocytochemistry was used to identify cells that contain OXA. Double labeling was made possible by using primary antibodies raised in different species in conjunction with species-specific secondary antibodies [donkey anti-rabbit, donkey anti-goat, donkey anti-mouse, or donkey anti-rat (Jackson ImmunoResearch Laboratories; diluted 1:200] coupled to contrasting fluorophores (FITC or Cy-3, as above). Reagents used to locate antigens simultaneously with OXA included antibodies to choline acetyltransferase (ChAT), CCK, 5-hydroxytryptamine (5-HT), substance P (SP), and vasoactive intestinal polypeptide (VIP). The sources of these antibodies and the evidence for their specificity are cited in Table 1. To enhance visualization of peptide immunoreactivity in nerve cell bodies, rats were injected with colchicine (5 mg/kg ip). Twenty-four hours later, the rats were killed as described above. Whole mounts of duodenum were fixed and stained from three different animals; cells in 50 ganglia were counted from each preparation from each rat. Data are expressed as means ± SE.

Preparations were examined by using a Radiance 2000 laser scanning confocal microscope (BioRad, San Fransisco, CA) attached to an Axioskope 2 microscope (Carl Zeiss, Thornwood, NY). Images of 512 × 512 pixels were obtained and processed by using Adobe Photoshop 6.0 (Adobe Systems, Mountain View, CA) and printed with a Kodak (XLS-8600) printer.

Electromyography. Animals were anesthetized with pentobarbital sodium (50 mg/kg ip; Apoteksbolaget, Umeå, Sweden), and through a midline incision, three bipolar stainless steel electrodes (SS-5T, Clark Electromedical Instruments, Reading, UK) were implanted into the muscular wall of the small intestine 5 (D), 15 (J1), and 25 (J2) cm distal to the pylorus. A jugular vein catheter for administration of drugs was placed in all animals in a separate surgical session. The electrodes and catheters were tunneled subcutaneously to exit at the back of the animal’s neck. The animals were housed singly after surgery and allowed to recover for at least 7 days before experiments were undertaken. During the periods of recovery, rats were trained to accept experimental conditions. Experiments were carried out in conscious animals after an 18-h fasting period in wire-bottomed cages with free access to water. The rats were placed in Bollman cages, and the electrodes were connected to an electroencephalogram preamplifier (7P5B) operating a Grass polygraph 7B (Grass Instruments, Quincy, MA). The time constant was set at 0.015 s, and the low and high cutoff frequencies were set at 10 and 35 Hz, respectively.

Fasting motility. All experiments started with a control recording of basal myoelectric activity, during which four activity fronts of the migrating myoelectric complex (MMC) propagated over all three recording sites during a period of 1 h was observed. Infusion of OXA or OXB (Peninsula Laboratories, Merseyside, UK) was started immediately after the first activity front had passed the first electrode site, using a microinjection pump (CMA 100, Carnegie Medicine, Stockholm, Sweden). Either OXA or OXB, at doses of 100 and 500 pmol·kg⁻¹·min⁻¹ iv, was administered for 60 min, and the effect on small bowel motility was recorded.

Data and statistical analysis. The main characteristic feature of myoelectric activity of the small intestine in the fasted state, the activity front (phase III) of the MMC, was defined as a period of clearly distinguishable intense spiking activity with an amplitude at least twice that of the preceding baseline propagating aborally through the whole recording segment and followed by a period of quiescence. The MMC cycle length, duration, and propagation velocity of the activity fronts were calculated (4) as means of the 60-min study period. When no activity front was observed during the 60-min infusion period, a value of 61 min was given for that experiment. When only one or two activity fronts were observed followed by a long period of quiescence, the MMC cycle length was calculated from the activity front preceding the start of the infusion to the phase III observed and then from the observed activity front during the infusion to the end of the infusion period + 1 min. Data are expressed as median (range), unless otherwise stated. The data were assessed for statistical significance using the Mann-Whitney U-test or Wilcoxon’s signed-rank test at P < 0.05, as appropriate.

Table 1. Primary antisera used

<table>
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<th>Antiserum</th>
<th>Host</th>
<th>Dilution</th>
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<tr>
<td>OXA</td>
<td>rabbit</td>
<td>1:1,500</td>
<td>Alpha Diagnostic International (San Antonio, TX)</td>
</tr>
<tr>
<td></td>
<td>rabbit</td>
<td>1:1,500</td>
<td>Chemicon International (Temecula, CA)</td>
</tr>
<tr>
<td></td>
<td>goat</td>
<td>1:2,000</td>
<td>Santa Cruz Biotechnology (Santa Cruz, CA)</td>
</tr>
<tr>
<td>OX1R</td>
<td>rabbit</td>
<td>1:1,000</td>
<td>Alpha Diagnostic International</td>
</tr>
<tr>
<td>OX2R</td>
<td>rabbit</td>
<td>1:1,000</td>
<td>Alpha Diagnostic International</td>
</tr>
<tr>
<td>ChAT</td>
<td>goat</td>
<td>1:100</td>
<td>Chemicon International</td>
</tr>
<tr>
<td>CCK</td>
<td>rabbit</td>
<td>1:8,000</td>
<td>Chemicon International</td>
</tr>
<tr>
<td>5-HT</td>
<td>mouse</td>
<td>1:5,000</td>
<td>Dako Corporation (Carpinteria, CA)</td>
</tr>
<tr>
<td>SP</td>
<td>rat</td>
<td>1:1,000</td>
<td>Accurate Chemical (Westbury, NY)</td>
</tr>
<tr>
<td>VIP</td>
<td>mouse</td>
<td>1:500</td>
<td>CURE/Gastroenteric Biology Center (Antibody/RIA Core, National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-41301)</td>
</tr>
</tbody>
</table>

OXA, orexin A; OX1R, orexin receptor 1; OX2R, orexin receptor 2; ChAT, choline acetyltransferase; 5-HT, 5-hydroxytryptamine; SP, substance P; VIP, vasoactive intestinal polypeptide.
RESULTS

OXA-like immunoreactivity in the duodenum. OXA-immunoreactivity was predominantly found in nerve fibers in the duodenum. Numerous OXA-containing neurites were present in submucosal (Fig. 1A) and myenteric (Fig. 1B) ganglia and in the interconnecting nerve strands. These processes were varicose and frequently encircled neuronal cell bodies (Fig. 1B). OXA nerve fibers were also observed in the longitudinal (Fig. 1C) and circular (Fig. 1D) muscle, running parallel to the muscle cells, and in extremely thin nerve fibers in the mucosa (not illustrated). Innervation of the mucosa was sparse compared with the dense OXA innervation of the ganglia and muscle.

Both neuronal cell bodies and endocrine cells displayed OXA immunoreactivity. OXA-immunoreactive neurons were found in submucosal (Fig. 1A) and myenteric (Fig. 1B) ganglia. The immunoreactivity in the cytoplasm of such cells was relatively low but was higher in colchicine-treated animals (14). No immunoreactivity was found in control sections that were processed without the primary antisera (not illustrated). The number of OXA-positive nerve cells in submucosal ganglia ranged from 1 to 14; the average was 6.0 ± 1.1 (n = 50 ganglia in preparations from 3 animals). The number of OXA-positive nerve cells in myenteric ganglia ranged from 1 to 9; the average was 5.3 ± 0.4 (n = 50 ganglia in preparations from 3 animals).

Further studies were done to identify neurochemically the neurons with OXA-like immunoreactivity in the duodenum. All OXA-immunoreactive submucosal neurons (Fig. 2A) contained ChAT (Fig. 2B), the synthesizing enzyme for acetylcholine. Thus these cells had a cholinergic phenotype. OXA (Fig. 2C) was also found in neurons that displayed VIP immunoreactivity (Fig. 2D). All VIPergic neurons contained OXA; however, ~25% of OXA-immunoreactive neurons had VIP. OXA-immunoreactive nerve fibers in the deep layer of the circular muscle (Fig. 2E) also contained VIP (Fig. 2F).

As in the submucosal plexus, OXA neurons in the myenteric plexus (Fig. 3, A and C) displayed ChAT immunoreactivity (Fig. 3, B and D) and OXA/ChAT-immunoreactive varicosities frequently surrounded nonreactive cell bodies (Fig. 3, E and F). In addition, a subset of OXA-immunoreactive neurons (Fig. 3G) and nerve fibers displayed VIP immunoreactivity (Fig. 3H).

In contrast to OXA neurons, OXA-immunoreactive endocrine cells were intensely stained (Fig. 4A) and appeared to be more numerous than OXA neurons. OXA-immunoreactive endocrine cells were found in crypts and scattered among the epithelial cells along the villi. Immunoreactivity was localized in granules at the base of cells, occasionally in proximity to OXA-immunoreactive nerve fibers (Fig. 4B). OXA-immunoreactive endocrine cells did not contain CCK (not illustrated); however, a subset displayed 5-HT (Fig. 4, C and D) and SP (Fig. 4, E and F) immunoreactivity. Thus OXA is co-stored with 5-HT in enterochromaffin (EC) cells.

Orexin receptor-like immunoreactivity in the duodenum. Sites of orexin receptor-like immunoreactivity were found in proximity to orexin-containing nerve fibers (Fig. 2A).

Fig. 1. Orexin A (OXA)-like immunoreactivity in the rat duodenum. OXA-like immunoreactivity is found in the cytoplasm of a subset of nerve cell bodies in the submucosal (A) and myenteric (B) plexus (arrow). Varicose nerve fibers are abundant in the interganglionic connectives and ganglia, where they frequently encircle lightly labeled OXA neurons (B, arrowhead). Varicose OXA-containing nerve fibers are found in the longitudinal (C) and circular muscle (D). Labeled fibers travel parallel to the muscle cells. Markers, 20 μm.
fibers. OX1R immunoreactivity was displayed by a large group of submucosal (Fig. 5, A and B) and myenteric (Fig. 5, D and E) neurons, many of which were contacted by OXA-immunoreactive varicosities (Fig. 5E). In general, immunolabeling was cytoplasmic, filling the perikarya and occasionally the proximal dendrites of a subset of enteric neurons. The cytoplasmic localization of OX1R in enteric neurons agrees with previous studies that have visualized these receptors in fixed and permeabilized cells, using COOH-terminal antibodies (3, 14). At least part of the internal labeling may represent receptors in transport to and from the cell membrane. The number of OX1R-positive nerve cells in submucosal ganglia ranged from 1 to 14; the average was 6.3 ± 1.4 (n = 50 ganglia in preparations from 3 animals). The number of OX1R-positive nerve cells in myenteric ganglia ranged from 3 to 26; the average was 14.3 ± 5.2 (n = 50 ganglia in preparations from 3 animals).

OXR1 immunoreactivity was also displayed by nerve fibers surrounding the mucosal crypts (Fig. 5B) and extending to the tip of the villi and “flower spray” endings in the subepithelial plexus (Fig. 5C). In addition, nerve fibers displayed OX1R immunoreactivity in the circular muscle (Fig. 5F). In contrast to the localization of OX1R in the ENS, only enteroendocrine cells expressed OX2R (Fig. 6A), a subset of which contained 5-HT (Fig. 6, B and C).

**Effect of orexins on the MMC.** Infusion of OXA and OXB had an inhibitory effect on the MMC. OXA increased the MMC cycle length in a dose-dependent fashion [15.6 (11.4–23.4), 31.9 (25.3–47.2), 39.5 (25.3–61) min for saline and OXA 100 and 500 pmol·kg⁻¹·min⁻¹, respectively (both P < 0.05 at the J2
level). No effect was seen on the duration or propagation velocity of activity fronts of the MMC through the gut segment under study (Table 2 and Fig. 7).

The MMC cycle length was substantially prolonged also by infusion of OXB. Compared with saline, the MMC cycle length was prolonged from 12.7 (10.6–30.1) to 32.9 (21.6–55.5; P < 0.05) and 27.8 (23.3–61.0) min by OXB at 100 and 500 pmol·kg⁻¹·min⁻¹, respectively. Again, there was no effect on the duration and propagation velocity of the activity fronts during OXB infusion (Table 2 and Fig. 7).

**DISCUSSION**

This study demonstrates, for the first time, an effect of peripherally administered orexins on the MMC, and it investigates the distribution of orexins and orexin receptors in nerve circuits within the rat duodenum, commonly the origin of the MMC. The distribution of OXA and orexin receptor immunoreactivity in the rat duodenum confirms and extends our previous findings demonstrating the expression of orexins and orexin receptors in the ileum of several species, including humans (14).

In the rat duodenum, OXA immunoreactivity occurred in both nerve cell bodies and endocrine cells and nerve fibers that innervated myenteric and submucosal ganglia and the circular muscle. Subsets of OXA-immunoreactive neurons were observed in each plexus. Most OXA-immunoreactive neurons contained ChAT, and a subset was VIP immunoreactive. ChAT immu-
noreactivity is found in nearly all nerve cell bodies in the submucosal plexus of the rat ileum, and in ~75% of nerve cell bodies in the myenteric plexus (18). The coincident localization of ChAT in OXA-immunoreactive neurons suggests that these neurons are likely to be excitatory, because acetylcholine is an excitatory neurotransmitter.

About 45% of submucosal nerve cells in the rat ileum contain VIP (18). These cells include secretomotor neurons and interneurons projecting within the submucosal plexus and to neurons in the myenteric plexus (7, 22). Myenteric VIP cells are thought to be inhibitory motoneurons that mediate relaxation of the circular muscle (9). The co-storage of VIP and OXA immunoreactivities thus implies that the OXA-immunoreactive neurons are secretomotor neurons, interneurons, and/or inhibitory motorneurons. The mucosa contained OXA-immunoreactive nerve fibers; however, the innervation of the mucosa was sparse compared with the dense OXA innervation of ganglia and muscle. Thus OXA neurons are more likely to be interneurons and/or motoneurons. Furthermore, the presence of OXA in VIPergic nerve fibers innervating the circular muscle suggests that orexin might modulate small intestinal motility.

OXA was observed in numerous endocrine cells in the mucosa. OXA-containing endocrine cells were found in crypts and scattered among the epithelial cells along the villi. A subset of these cells displayed 5-HT and SP immunoreactivity. Thus OXA is found in EC cells.

5-HT is released from EC cells in response to mucosal stimulation and activates both intrinsic (15, 16) and extrinsic (12) primary afferent neurons, resulting in reflex alteration of gut function. 5-HT has also been
shown to participate in the initiation of the peristaltic reflex (28). The presence of OXA in EC cells suggests that the peptide could have a function similar to 5-HT. Furthermore, because EC cells appear to express OX2R, orexins may be able to modulate 5-HT release and possibly regulate their own release in an autocrine-like fashion.

It is not yet known whether OXA is released from EC cells; however, OXA increases peristalsis in the isolated guinea pig distal colon (14). Moreover, an extensive array of OX1R-immunoreactive nerve fibers was found in the lamina propria, just beneath the epithelium. It thus seems reasonable to propose that OXA is released from EC cells, which then activates OX1R on

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Fig. 5. Orexin receptor 1 (OX1R)-like immunoreactivity in the rat duodenum. A and B: OX1R immunoreactivity is displayed by submucosal nerve cell bodies (arrowheads) and processes that enter interganglionic connectives and encircle (arrow, B) mucosal crypts (c). C: OX1R immunoreactivity forms “flower spray” endings (arrow, C) along nerve fibers in the subepithelial plexus. D and E: OX1R-immunoreactive neurons are abundant in the myenteric plexus (D). The soma of a subset of these cells is contacted by OXA-immunoreactive varicosities (arrows, E). F: OX1R is expressed by nerve fibers in the circular muscle. Markers, 15 μm (A, B); 10 μm (C); 20 μm (D-F).

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Fig. 6. Orexin receptor 2 (OX2R)-like immunoreactivity in mucosal endocrine cells. A: OX2R is expressed by an endocrine cell in a duodenal crypt. OX2R immunoreactivity (B) is displayed by a 5-HT-containing EC cell (C). Markers, 5 μm.
the mucosal processes of intrinsic primary afferent neurons. Once activated by OXA, therefore, these cells are able to modulate reflexes by synapsing with motor neurons (directly or via interneurons) in the myenteric plexus. The mucosa is also innervated by primary afferent neurons of vagal and spinal origin (11). Thus it is possible that OXA activates OX1R found on the mucosal processes of extrinsic primary afferent neurons. In support of this idea, OX1R immunoreactivity was

Table 2. Effect of OXA and OXB on fasting motility in the rat (n = 8)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control OXA</th>
<th>OXA 100, pmol·kg⁻¹·min⁻¹</th>
<th>OXA 500, pmol·kg⁻¹·min⁻¹</th>
<th>Control OXB</th>
<th>OXB 100, pmol·kg⁻¹·min⁻¹</th>
<th>OXB 500, pmol·kg⁻¹·min⁻¹</th>
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<td></td>
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<tr>
<td>D</td>
<td>16.3(8.9–21.8)</td>
<td>21.3(15.7–40.7)*</td>
<td>26.9(18.5–61)‡</td>
<td>15.3(11.1–27.0)</td>
<td>21.2(13.6–44.0)*</td>
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<tr>
<td>J1</td>
<td>15.9(12.4–21.9)</td>
<td>25.6(19.8–44.5)*</td>
<td>30.9(21.8–61)*</td>
<td>15.2(10.6–29.7)</td>
<td>25.5(18.0–45.2)*</td>
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<td>J2</td>
<td>15.6(11.4–23.4)</td>
<td>31.9(25.3–47.2)*</td>
<td>39.5(25.3–61)‡</td>
<td>12.7(10.6–30.1)</td>
<td>32.9(21.6–55.5)*</td>
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<td>D–J1</td>
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<td>1.5(1.0–2.2)</td>
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<td>1.8(1.1–2.3)</td>
<td>1.4(0.8–2.0)</td>
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Data shown as median (range). D, duodenum 5 cm distal to pylorus; J1, jejunum 15 cm distal to pylorus; J2, jejunum 25 cm distal to pylorus. When no activity fronts were present during 60 min infusion, an arbitrary value of 61 min was given. *P < 0.05 vs. control and (†) vs. 100 pmol·kg⁻¹·min⁻¹.

Fig. 7. Effect of orexins on fasting small bowel motility. Representative electromyographical recording of fasted small bowel motility in the rat during saline, OXA, or orexin B infusion during 60 min. D, duodenum 5 cm distal to pylorus; J1, jejunum 15 cm distal to pylorus; J2, jejunum 25 cm distal to pylorus.
played by flower spray endings in the subepithelial plexus. Flower spray endings are typically formed by vagal afferents (26). Moreover, both dorsal root (3) and nodose ganglion (unpublished data) neurons have recently been shown to display OXA and OX1R immunoreactivity. Thus OXA, like 5-HT and several other gut hormones (27), can modulate gastrointestinal motility. In addition, OXA appears to play a role in sensory transduction in the gastrointestinal mucosa.

To investigate whether peripheral orexins specifically regulate motility of the small intestine, we examined the effects of intravenous infusion of orexins on the MMC. Intravenous infusions of OXA and OXB at doses of 100 pmol·kg⁻¹·min⁻¹ increased the MMC cycle length without affecting the duration or the propagating velocity of the activity fronts. At higher infusion rates (500 pmol·kg⁻¹·min⁻¹), only OXA further increased the MMC cycle length. Although only a few points along the dose–response curve of the orexins were studied, data suggest that greater plateaus of the dose response and maximal efficacy were achieved with lower doses of OXB than with OXA. This would possibly indicate OXB and its preferred receptor mechanism as the more important of the two under physiological conditions. Of interest is the fact that intravenous infusions of orexins produced a comparable inhibitory effect to VIP on the MMC (17). Because OXA nerve fibers in the circular muscle of the duodenum also contain VIP, the inhibitory effect of orexins on the MMC may be mediated by VIP or at least involve similar mechanisms as VIP.

The present results suggest that orexins modulate duodenal motility through peripheral actions of the peptides; however, this study cannot precisely locate the site of the peptide’s action. OX1R-like immunoreactivity is displayed not only by enteric neurons, but also nerve fibers in the mucosa and circular muscle; therefore, OXA could modulate motility by acting on receptors located at nerve synapses within the enteric plexuses, mucosa, and/or muscle. In addition, because endocrine cells of the mucosa express OX2R, the effect of OXB may be mediated through the EC cell, which contains possible MMC regulatory peptides.

We have previously shown that superfusion of OXA enhances motility in the isolated guinea pig distal colon (14). We have now demonstrated that intravenous infusions of the peptide inhibit the MMC in the small intestine of rats, resulting in a more fedlike motor pattern. Thus the effects of orexins on motility are likely to be exerted through interactions with different receptor subtypes on nerves and endocrine cells in the gut. Future studies employing selective orexin receptor antagonists would greatly improve our understanding of the function of OX1R and OX2R in gut motility.

In summary, we have characterized the distribution of orexins and orexin receptors in the duodenum and showed that the peptides inhibit fasting motility in the rat. On the basis of our findings, it seems likely that the orexins may play a significant role in modulating gastrointestinal function and thus may be involved in the processing of nutrients in addition to the central stimulation of food intake.

This study was supported by grants from National Institute of Neurological Disorders and Stroke (NS-27645), The American Diabetes Association (to A. L. Kirchgessner), The Swedish Research Council, the Swedish Medical Society, Funds of the Karolinska Institutet, the Professor Nanna Svartz Fund, the Magnus Bergvall Fund, the Tore Nilsson Fund, the Ruth and Richard Juhlin Fund, the Arbetarvarelendes Porslönengar-sjukforsäkring Jubilee Foundation for Research in National Diseases, Jeanssons Foundation, Bengt Ihre Foundation, STINT Swedish Foundation for International Cooperation in Research and higher education, and the Johan Thorne Holst Foundation.

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