Orexins in the Brain-Gut Axis

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Orexins (hypocretins) are a novel pair of neuropeptides implicated in the regulation of energy balances and arousal. Previous reports have indicated that orexins are produced only in the lateral hypothalamic area, although orexin-containing nerve fibers were observed throughout the neuraxis. Recent evidence shows that orexins and functional orexin receptors are found in the periphery. Vagal and spinal primary afferent neurons, enteric neurons, and endocrine cells in both the gut and pancreas display orexin- and orexin receptor-like immunoreactivity. Orexins excite secretomotor neurons in the guinea pig gut and modulate gastric and intestinal motility and secretion. In addition, orexins modulate hormone release from pancreatic endocrine cells. Moreover, fasting up-regulates the phosphorylated form of cAMP response element binding protein in orexin-immunoreactive enteric neurons, indicating a functional response to food status in these cells. The purpose of this article is to summarize evidence for the existence of a brain-gut network of orexin-containing cells that appears to play a role in the acute regulation of energy homeostasis. (Endocrine Reviews 23: 1-15, 2002)

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I. Introduction

The orexins (1), also called hypocretins (2), were first described just 4 yr ago; yet more than 280 papers on the peptides have already been published. Interest in the orexins began with the observation that these novel neuropeptides are produced by a small group of neurons in the perifornical and lateral hypothalamic area [LHA (1-4)], a region classically implicated in the control of mammalian feeding behavior (5-7). Intracerebroventricular administration (1, 8-10), or direct injection of orexins into the LHA (11), has been shown to increase food intake in rodents in a dose-dependent manner. Conversely, an orexin receptor antibody inhibits food intake in fasted rats, indicating that endogenous orexins are necessary for feeding (12). Furthermore, orexin mRNA expression is up-regulated by fasting (1, 13), suggesting that these neurons become activated under conditions of hunger.

Orexin neurons project within the hypothalamus and throughout the central nervous system (CNS) to nuclei known to be important in the control of feeding (3, 4, 14, 15). In addition, abundant orexin nerve fibers and orexin receptors are found in nuclei concerned with maintenance of wakefulness (14, 15), and several reports implicate a dys-function of the orexin system in human and canine narcolepsy (16-18). Recently, genetic ablation of orexin neurons in mice was shown to result in narcolepsy, hypophagia, and obesity (19). This finding confirms the importance of hypothalamic orexin-containing neurons in the regulation of sleep/wake states and further suggests a role in energy metabolism.

Although the hypothalamus has received considerable attention regarding energy homeostasis, the gut also participates in the regulation of food intake. The presence of food in the bowel, through activation of chemo- and mechanoe-sensitive endings, stimulates the release of several regulatory peptides that control gut motility and secretion (20, 21). Several of these peptides [for example, cholecystokinin (CCK)] also act as feedback “satiety” signals responsible for termination of a meal (21-23). Satiety signals are inhibited during fasting, and replaced by “hunger”-related signals, also from the gut, that may be enhanced by low plasma concentrations of glucose (21).

The enteric nervous system (ENS), which is composed of neurons that reside within the wall of the gastrointestinal tract and contains as many neurons as the entire spinal cord (24), directly senses, integrates, and regulates the machinery
II. Discovery of the Orexins

The orexins were discovered during a search for endogenous ligands that activate orphan G protein-coupled receptors (1). Using more than 50 cell lines, each expressing a distinct orphan G protein-coupled receptor cDNA, Sakurai et al. (1) tested the ability of HPLC fractions of rat brain extracts, to increase intracellular calcium concentrations ([Ca$^{2+}$]). The investigators discovered several HPLC fractions that elicited a robust increase in [Ca$^{2+}$], in a human embryonic kidney (HEK293) cell line expressing a receptor originally termed HFGAN72. This receptor was initially identified as an expressed sequence tag from human brain. When the major peak of bioactivity in the fractions was purified to homogeneity and sequenced, a novel peptide of 33 amino acids in length, with an N-terminal pyroglutamyl residue and an amidated C terminus, was discovered. The peptide, termed orexin-A, also contained two intrachain disulfide bonds, and sequencing of similar extracts from bovine brain revealed exact interspecies homology.

In addition to orexin-A, the HPLC fractions contained two minor peaks of activity, designated B and B'. Peak B consisted of a 28-amino acid peptide, termed orexin-B, which also possessed an amidated C terminus and was 46% identical in sequence to orexin-A. Peak B' consisted of an N-terminally truncated orexin-B, which was termed orexin-B (3–28). Further analysis revealed that both orexin-A and orexin-B were derived from the same 130-amino acid precursor, rat prepro-orexin, by proteolytic processing. Human and mouse prepro-orexin sequences were determined and found to be 83% and 95% identical to their rat counterparts, respectively. Radiation hybrid mapping showed that the human prepro-orexin gene maps to a locus at chromosome 17q21. Thus, the prepro-orexin gene has been proposed to be a candidate gene for a group of neurodegenerative disorders called “chromosome 17-linked dementia” (40).

Independently, de Lecea et al. (2), using directional tag PCR subtraction, identified a hypothalamic-specific mRNA encoding a precursor protein that they called prepro-hypocretin and predicted that processing of this prepro-peptide would yield two peptides, one of 39 and another of 29 amino acids. Because the cell bodies that expressed this gene were thought to be located exclusively in the hypothalamus, and because of a weak homology to the gut peptide secretin, the peptides were named hypocretin-1 and hypocretin-2. Subsequent comparisons revealed that prepro-orexin and pro-hypocretin were the same gene, and that hypocretin-1 and hypocretin-2 had sequences in common with orexin-A and -B, respectively (15, 37). Thus, orexin and hypocretin are the same molecule. Nevertheless, in this article, the term orexins will be used to denote the orexin/hypocretin peptides, since orexin immunoreactivity and orexin mRNA expression have been found outside the hypothalamus (1, 26, 41).

III. Characterization of the Orexin Receptors

In their remarkable paper, Sakurai et al. (1) not only reported the structure of the orexins, but also identified the amino acid sequences of the receptors for the two peptides. The original HFGAN72 receptor, subsequently called OX1R, was shown to bind orexin-A with high affinity and bind orexin-B with 100- to 1,000-fold lower affinity. However, a related receptor, OX2R, identified by searching database entries with the OX1R sequence, was demonstrated to have equally high affinities for both peptides. Thus, OX2R was concluded to be a nonselective receptor for both orexin-A and -B peptides, while OX1R was concluded to be moderately selective for orexin-A. The binding of both ligands to either receptor was associated with changes in intracellular calcium concentrations. Evidence from receptor-expressing cells suggests that OX1R is coupled exclusively to the Gq subclass of G proteins, whereas OX2R may couple to Gi/o and/or Gq (1, 15, 42).
IV. The Orexin System in the Brain

Immunohistochemical and in situ hybridization studies have shown that in the CNS, orexin-producing cells are restricted to a few nuclei in the hypothalamus, including the perifornical nucleus, the LHA, and the dorsomedial hypothalamic nucleus (Fig. 1 and Refs. 1–4, 43, and 44). Orexin neurons are organized bilaterally and symmetrically and have been observed in all species investigated so far, including bovine, guinea-pig (A. L. Kirchgessner, unpublished observations), hamster, human, monkey, mouse, rat, and frog (Fig. 1 and Refs. 1–4 and 43–48). Approximately 1,100 orexin-containing cell bodies have been estimated to be present in the rat brain, using an antibody to prepro-orexin (14). Despite their highly restricted origin, orexin nerve fibers ramify widely throughout the CNS, with particularly abundant projections found in the olfactory bulb, cerebral cortex, thalamus, hypothalamus, brainstem, and all levels of the spinal cord (3, 4, 13–15, 45–49). The widespread projections of the orexin neurons throughout the neuroaxis suggest that activation of orexin circuits probably modulates a variety of systems (14, 15), including those involved in the regulation of food intake. The fact that orexins can increase the release of either excitatory or inhibitory neurotransmitters, by acting directly on axon terminals (2, 42), indicates that the peptides could ultimately increase or decrease the activity of innervated brain circuits.

A. Hypothalamus

Within the hypothalamus, orexin neurons project to the arcuate nucleus (3, 4) and specifically innervate NPY-containing cell bodies (46). Reciprocal connections from NPY neurons to orexin neurons in the LHA have also been identified (43, 45, 46). In addition, orexin-containing nerve fibers terminate in close apposition to NPY-immunoreactive nerve terminals in the paraventricular nucleus [PVN (45, 46)]. NPY is a potent orexigenic peptide that is released in the PVN and surrounding sites to stimulate feeding (50, 51). Since arcuate NPY neurons are excited by orexins (15, 42), probably through the activation of an OX1R Gq-coupled pathway (15), this suggests that orexin-stimulated feeding might occur through NPY pathways (4, 52). Both NPY Y1 and Y5 receptor antagonists reduce orexin-stimulated feeding (53, 54). Thus, activation of NPY-containing feeding pathways is at least partially responsible for the effects of orexin on food intake.

B. Dorsal vagal complex

In the hindbrain, orexin-immunoreactive fibers are found in the dorsal vagal complex, comprising the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (DMV), and the area postrema (3, 15). The NTS relays vagally transmitted afferent signals from the gut that are related to feeding (21, 23). The NTS also contains glucosensitive neurons that respond with altered electrical activity to changes in blood glucose and the presence of food in the gut (55, 56). The DMV consists of motor neurons that control gut motility and the secretory responses that are especially important for digestion. The DMV is also responsible for the initiation of cephalic-phase responses that prepare the gut for the arrival and subsequent digestion of nutrients (57). The area postrema, which is interconnected with the dorsal vagal complex, is an important circumventricular organ through which circulating systemic factors, such as gut peptides and glucose, can gain access to the brain (58).

Although the types of neurons in the dorsal vagal complex that are innervated by orexin-containing nerve terminals have not been identified, it is likely that orexins alter the activity of vagal motor neurons and/or modulate the response of NTS neurons to gastrointestinal stimuli. It has been demonstrated that stimulation of the LHA excites neurons in the DMV (59) and increases the activity of vagal efferents.
(60). Thus, it is reasonable to expect that modulation of vagal activity by orexins could influence cephalic phase reflexes and/or affect gastrointestinal motility and secretion. Furthermore, since neurons in the NTS project back to the LHA (61), orexin neurons may be regulated by satiety signals relayed through the NTS.

C. Distribution of orexin receptors

In parallel to the diffuse orexin-containing projections from the LHA, in situ hybridization studies with orexin receptor riboprobes demonstrate that orexin receptors are expressed in a pattern consistent with orexin nerve fibers (62–65). However, the expression patterns for OX1R and OX2R are strikingly different. Within the hypothalamus, OX1R mRNA is most abundant in the dorsomedial portion of the ventromedial hypothalamic nucleus (VMH). Dense expression of OX1R is also found in the anterior hypothalamic area just dorsal to the suprachiasmatic nucleus. In contrast, OX2R mRNA is expressed in many hypothalamic nuclei including the tuberomammillary nucleus, the LHA, the arcuate nucleus, and PVN (63, 65). The tuberomammillary nucleus is the only source of histamine in the CNS. Since histamine is crucial for the maintenance of wakefulness (66), OX2R in this region has been postulated to play a role in the regulation of sleep/wake states (65).

The expression of orexin receptors in the VMH, LHA, arcuate nucleus, and PVN is consistent with a role of hypothalamic orexin systems in regulating food intake. The VMH is strongly implicated in the regulation of food intake since its destruction causes obesity (67). The PVN is the site of action of many orexigenic agents including NPY (50, 51) and galanin (68). The PVN is also involved in the regulation of gut functions via its projection to the dorsal vagal complex (69). For example, stimulation of the PVN evokes an increase in gastric acid secretion (70) and a transient increase in motility (71).

Surprisingly, little orexin receptor mRNA has been detected in the NTS and DMV (65), although these regions appear to receive a moderately dense orexin innervation (3, 15). Locations with dense orexin immunoreactivity but little receptor mRNA may reflect presynaptic innervation on axon terminals (42), whose cell bodies are located at some distances. Recent findings support a presynaptic action of orexin-A in the DMV (72). However, a postsynaptic action on DMV neurons via OX1R receptors has also been demonstrated and appears to mediate the stimulatory effects of central orexin-A on gastric motility (72).

Other brain areas that display relatively dense expression of OX1R include the CA1 and CA2 regions of the hippocampus, raphe nuclei, and the locus coeruleus (62–65). The locus coeruleus and dorsal/median raphe nuclei are major centers for the noradrenergic and serotonergic neurons, respectively. High levels of OX1R expression in these nuclei suggest a regulatory role of orexins on the monoaminergic systems. On the other hand, OX2R mRNA is also present in basal forebrain structures (amygdala and bed nucleus of the stria terminalis), linked to such functions as memory storage and attention, and the nucleus accumbens (62–65). The nucleus accumbens is the major recipient of the mesolimbic dopaminergic projection and serves a key role in brain reward mechanisms, which may mediate the positive reinforcing effect of food (73).

V. Orexins and the Regulation of Feeding Behavior

A. Orexins increase food intake

Sakurai et al. (1) initially examined the effects of the orexins on feeding behavior, because the mRNA for the precursor of these peptides was abundantly expressed in the LHA, a region classically implicated in the regulation of both food intake and metabolism (5–7). In fact, Anand and Brobeck (74) called the LHA a “feeding center,” since lesions of the LHA caused substantial reductions in food intake and body weight leading to starvation if the animals were not force-fed (7). The fact that electrical stimulation of the LHA produced vigorous feeding, leading to an increase in body weight (7), suggested that stimulation activated an orexigenic pathway originating within or in the vicinity of the LHA.

Sakurai et al. (1) demonstrated that intracerebroventricular administration of orexin-A or orexin-B in rats increased food intake in a dose-dependent manner, with orexin-A significantly more effective than orexin-B, possibly due to activation of both OX1R and OX2R subtypes (1). Based on these findings, the orexins were named after the Greek word orexis, which means appetite (1). Subsequently, orexin-A has been reported to increase food intake in several species (8–10, 15, 75), including goldfish (76), and after microinjections in several hypothalamic nuclei, including the PVN, dorsomedial hypothalamic nucleus, LHA, and perifornical area (11, 14, 15). In addition, Yamada et al. (12) reported a profound inhibition of natural feeding in fasted rats by central injection of an antiorexin-A antibody. Furthermore, a selective OX1R receptor antagonist, SB-334867-A, has been shown to inhibit spontaneous nighttime feeding over several days, as well as orexin-A-induced feeding, and (over the first 4 h) feeding stimulated by an overnight fast (77, 78). Thus, endogenous orexins and stimulation of the OX1R receptor appear to be necessary for normal feeding.

In contrast to orexin-A, the results obtained with orexin-B have been more variable; therefore, orexin-B has been concluded to have little, if any, effect on feeding (8, 10, 11, 79). The lack of apparent feeding disturbances in OX2R mutant dogs further supports this conclusion (80); however, the moderately dense expression of OX2R in the arcuate, PVN, and LHA (65) suggests that conclusions regarding the specific receptor(s) involved in the feeding effects of orexins cannot be made without further study.

A comparative evaluation of the potency of orexins, administered intracerebroventricularly, with other hypothalamic orexigenic peptides has demonstrated that orexin-A is significantly less potent in stimulating food intake than NPY (8, 51). In addition, unlike NPY, chronic administration of orexin-A does not induce obesity in normal rats (81, 82). However, its duration of action is longer than that of NPY (1), and the magnitude of the effect of orexins is similar to that of other hypothalamic appetite-stimulating peptides, such as melanin concentrating hormone (MCH) and galanin (8). Interestingly, MCH and galanin also do not cause obesity in...
normal rats (83, 84). However, mice with targeted deletions of the MCH gene are hypophagic (85), as are orexin knockout mice (19). Thus, orexins appear to be involved in the short-term regulation of feeding, rather than the long-term maintenance of body weight.

Orexin-A appears to increase food intake by delaying behavioral satiety, i.e., the normal transition from eating through grooming to resting (75). In addition, results with chronic intracerebroventricular infusion of orexin-A over several days, suggest that the peptide also disrupts the normal circadian feeding pattern in rats by increasing daytime and decreasing nighttime food intake (52, 77, 79, 81). Metabolic effects of orexins have also been shown to be dependent on circadian phase (86). Interestingly, disruptions of normal circadian feeding patterns are well described effects of LHA lesions (7). In addition, a role for the LHA in the regulation of sleep-wakefulness has been established (6, 87). This suggests that increased arousal or prolonged wakefulness may contribute to orexin-A-stimulated food intake in continuously infused rats.

Orexin neurons innervate and activate brain areas that promote wakefulness, such as the aminergic locus coeruleus, which diffusely activates the cortex, and the tuberomammillary nucleus (14, 15, 88). Application of orexin-A increases the firing rates of aminergic neurons, in vitro (88) and suppresses rapid eye movement sleep in a dose-dependent manner (89). Furthermore, orexin knockout mice exhibit a phenotype strikingly similar to human narcolepsy (90), as do canines with a mutation of the OX2R gene (80). People with narcolepsy have chronic, sometimes severe, daytime sleepiness that is often accompanied by episodic intrusions of rapid eye movement sleep and sudden episodes of muscular paralysis or weakness known as cataplexy. These findings leave no doubt that orexin neurons play essential roles in the control of feeding and energy balance but also regulate wakefulness. This is of significance because, during periods of nutritional depletion, alertness may help to ensure survival. Clearly, in such circumstances, searching for food would be preferable to sleeping.

B. Orexin neurons respond to metabolic signals

Orexin neurons respond to several metabolic signals that reflect the state of energy resources. Hypothalamic prepro-orexin mRNA levels are increased significantly after 48 h of fasting and by acute (6 h) insulin-induced hypoglycemia (1, 13, 91), suggesting activation of these neurons under conditions of hunger. However, no changes in expression occurred when rats with acute or chronic insulin-induced hypoglycemia were allowed to eat (91, 92) or when they were given glucose to maintain euglycemia (93). In addition, no increases in hypothalamic prepro-orexin mRNA levels were seen in rats with increased appetite due to insulin-deficient diabetes, or access to palatable foods (91, 94). Thus, orexin neurons are not stimulated under all conditions of hunger. Based on these findings, Cai et al. (91) concluded that low plasma glucose levels and/or absence of food from the gut stimulates orexin neurons and postulated that orexins are involved in short-term feeding behavior. Furthermore, they suggested that orexin neurons might belong to a subset of LHA neurons that are stimulated by falls in serum glucose and inhibited by vagally transmitted satiety signals that are relayed through the NTS.

It is well known that a decline of blood glucose level can signal the initiation of food intake (95). Circulating glucose concentrations show a dip before the onset of most meals in human subjects and rodents. When the glucose dip is prevented, the next meal is delayed (21). Mayer’s (96) glucostatic theory of feeding postulates that eating occurs to maintain glucose availability. The LHA contains “glucosensitive” neurons that are activated by hypoglycemia and suppressed by elevated blood glucose, suggesting a role in short-term nutrient sensing. Glucosensitive neurons account for approximately 25% of LHA neurons (95, 97); therefore, at least some of the orexin-containing neurons may be glucosensitive. Recent studies support this suggestion. Approximately 30% of orexin-immunoreactive neurons were shown to display Fos-like immunoreactivity, a marker of neuronal activation, during insulin-induced hypoglycemia (92, 98). In addition, Shiraiishi et al. (99) demonstrated that orexin-A caused an increase in spike discharge in about 67% of glucosensitive LHA neurons that they tested. Thus, glucosensitive cells express excitatory orexin receptors. Interestingly, orexin-A inhibited the activity of glucoresponsive neurons found in the VMH. Gluocytesensitive neurons are excited by glucose, and stimulation of these cells has been postulated to contribute to the cessation of eating. The opposite effects of orexins on the activity of glucosensitive and glucoresponsive hypothalamic neurons are consistent with the antagonistic roles of the LHA and VMH in feeding regulation (7).

The dorsal vagal complex is another important component of orexin feeding circuits. Therefore, it is not surprising that neuronal activation of orexin neurons was accompanied by the appearance of Fos immunoreactivity in neurons in the NTS and adjacent DMV (92). The NTS relays information from vagal afferents, including glucoreceptors in the gut and liver. In addition, the NTS, like the LHA, contains glucosensitive cells that are stimulated by hypoglycemia (5, 55, 56). The similar pattern of Fos activity in the NTS and LHA during insulin-induced hypoglycemia led the authors to conclude that the signals that triggered orexin neurons might be relayed via the NTS, which has a major projection to the LHA (61). Thus, the NTS may be an important regulator of orexin neurons and their responses to changes in glucose availability and prandial signals.

Orexin-containing neurons may also be sensitive to leptin. Leptin is a protein product of the ob (obese) gene (100) that is secreted by adipocytes in proportion to fat stores. Exogenously administered leptin reduces body weight and can inhibit the increased feeding stimulated by several orexigenic peptides (51, 101). The arcuate nucleus is a major site of leptin-responsive neurons and is considered an important “satiety center” on the basis of lesioning studies (51). Leptin-mediated inhibition of arcuate NPY neurons, and excitation of neurons that coexpress the anorectic peptides, cocaine-and amphetamine-regulated transcript and POMC, are believed to underlie the suppression of appetite by leptin (15, 102).

Leptin receptor immunoreactivity and signal transducer and activator of transcription 3, a transcription factor acti-
vated by leptin, are found in orexin-containing cells (103). Beck and Richy (104) showed that chronic administration of leptin reduces orexin-A levels in the LHA. In addition, Lopez et al. (105) found that leptin could inhibit the increase in orexin gene expression induced by fasting. They also found increased OX1R mRNA expression with fasting that was suppressed by leptin treatment. Interestingly, no change in OX2R mRNA expression was detected in either fasted or leptin-treated conditions. Thus, it seems likely that orexin cells are modulated by leptin, probably via OX1R, and changes in orexin expression are involved in the response to fasting.

C. Orexins and cephalic phase reflexes

The anatomical and experimental data described above clearly imply that central orexins play a role in the regulation of feeding behavior. Although several lines of evidence suggest that the hypothalamus is the primary brain site targeted by the orexins, the projection from the NTS to the LHA also appears to be an important regulator of orexin neurons and their response to changes in glucose availability and prandial signals (92). As such, the NTS may be involved in triggering hunger and eating in response to hypoglycemia and perhaps in terminating feeding episodes.

Orexin fibers and receptors are also found in the DMV (3, 15, 65). This raises the possibility that the orexins may control vagal outflow to the gastrointestinal tract and modulate activities such as gastric acid secretion and/or motility. Orexin-A dose-dependently increases gastric acid secretion when given centrally and with an intact vagus nerve (106). Others have found that orexin-A potently increases gastric motility when applied to the DMV (72). These findings suggest a role for orexins in the brain-gut axis.

In 1895, Pavlov and Schumowa-Simanowskaja (107) demonstrated that sensory stimulation induced by sham feeding evokes gastric acid secretion. It is now well known that gastric acid secretion occurs as part of cephalic phase reflexes, which consist of simultaneous activation of gastric acid and pancreatic enzyme secretion, antroduodenal motility, release of pancreatic polypeptide and gastrin, and gallbladder contraction. Cephalic phase responses are provoked by the thought, sight, smell, taste, and chewing of food that has an appetizing effect in the subjects studied (108). These responses are important because they prime the secretory capability of the gut, increasing the efficiency of the subsequent gastric and intestinal phases of secretion that occur in response to a meal. The potent stimulating effect of orexin-A on gastric acid secretion and motility and its dependence on vagal cholinergic outflow to the stomach suggests that orexins may mediate cephalic phase reflexes (109). Other peptides that have been shown to evoke cephalic phase responses include TRH (109), NPY (110), and ghrelin (111). Interestingly, like orexin, both NPY (82) and ghrelin (31, 112) stimulate feeding, and both peptides are found in the gut. Thus, orexigenic gut peptides evoke cephalic phase responses.

VI. The Orexin System in the Gut

A. Characteristics of the ENS

The ENS is directly responsible for controlling the motility and secretory activity of the bowel (24, 25, 113). It consists of groups of nerve cell bodies (ganglia) arranged in two interconnected plexuses (Fig. 2). The myenteric ganglia form a single plexus between the longitudinal and circular smooth muscle layers and are mainly concerned with control of motility. In small animals, the submucosal ganglia form a single layer and are concerned mainly with control of transmucosal water and electrolyte transport and local blood flow. In larger mammals, the submucosal ganglia form layers that are identified as inner, outer, and, in some cases, intermediate plexuses (114). Some data suggest that the outer submucosal plexus (closest to the circular muscle) shares control of the circular muscle with the myenteric plexus. The musculature, mucosal epithelium, and vasculature are the gut’s effector systems. The ENS coordinates activity of the primary effectors to produce different patterns (digestive, interdigestive, peristaltic) of gut behavior (113).

The innervation of the bowel differs from that of other peripheral organs because the gut is able to manifest reflex activity in the absence of connections with the CNS (25). This activity is made possible by the presence within the bowel of microcircuits that contain the necessary primary afferent neurons and interneurons, as well as the excitatory and inhibitory motor neurons that innervate gastrointestinal smooth muscle and glands. The ENS also contains neurons that project out of the gut to send signals to other digestive organs.
organisms, for example to the pancreas (115, 116) and gallbladder (117). The complexity of the functions controlled by the ENS is reflected in an equally complex organization that resembles that of the CNS more than the remainder of the peripheral nervous system. Many different classes of neurotransmitters have been found in the ENS, including most of those known also to be present in the CNS. On this basis, the ENS is sometimes referred to as the “brain-in-the-gut” or enteric “minibrain” (113, 118).

Despite the ability of the ENS to function independently of control by the CNS, it does not normally do so. The CNS affects the motility and secretory activity of the bowel (118–120). In fact, abdominal pain, diarrhea, nausea, and altered food intake can all be manifestations of emotional or traumatic stress.

The gut receives input from the brain in efferent vagal fibers and descending pathways in the spinal cord that connect to sympathetic preganglionic neurons in the thoracolumbar region. Efferent vagal fibers form synapses with neurons in enteric ganglia to activate circuits, which ultimately affect the outflow of signals in motor neurons to the effector systems (121–123). When the effector system is the musculature, its innervation consists of both inhibitory and excitatory motor neurons that participate in reciprocal control. If the effector systems are gastric glands or digestive glands, the secretomotor neurons are excitatory and stimulate secretory behavior.

Extrinsic primary afferent neurons carry information about the state of the gastrointestinal tract to the CNS to influence feeding behavior. Vagal primary afferent neurons have cell bodies in the nodose ganglia and axons that reach the gut via the vagus nerves, and spinal primary afferent neurons have cell bodies in the dorsal root ganglia. The axons of spinal primary afferent neurons in the thoracic and lumbar regions pass through sympathetic ganglia to reach the gut via splanchnic and mesenteric nerves. The axons of most spinal primary afferent neurons with cell bodies in sacral ganglia follow the pelvic nerves to reach the colon and rectum. Direct recordings of extrinsic primary afferent neurons have revealed that they react to several types of stimuli, including chemical changes in the intestinal lumen, distension of the gut wall, mechanical distortion of the mucosa, and changes in osmolarity and temperature. Hormones may also affect afferent nerve endings; therefore, it is still not clear whether sensory afferents are directly activated by nutrients or secondarily by hormones that are released by nutrients. In addition, spinal afferent nerves also carry nociceptive information to the CNS.

**B. Distribution of orexins and orexin receptors**

As the ENS contains a number of neuropeptides that have been reported to stimulate or suppress food intake (29–35), we hypothesized that orexins might be present in the gut. If orexins were present in the ENS, then, as in other sites where these peptides exist, the enteric ganglia would be expected to contain neurons that displayed orexin immunoreactivity.

Orexin-like immunoreactivity was found in the ENS with a panel of antibodies raised against different sequences in prepro-orexin, orexin-A, and orexin-B (26). In addition, RT-PCR analysis revealed prepro-orexin and orexin receptor mRNA expression in the myenteric-plexus of the rat intestine. Orexin peptides were found in the ENS of a number of species (guinea pig, rat, mouse, and humans), suggesting that the orexin gene has been conserved over long periods of mammalian evolution. Orexin-immunoreactive cell bodies and nerve fibers were observed in all regions of the gut; however, the densest innervation was in the duodenum into which the stomach empties after a meal.

Interestingly, there are few reports on orexins in visceral organs. Northern blot analysis and in situ hybridization studies have detected orexin and orexin receptor mRNA expression almost exclusively in the CNS (1, 2). Nevertheless, Sakurai et al. (1) did find orexin mRNA expression in the testes, and Lopez et al. (124) demonstrated a strong level of OX1R and OX2R mRNA expression in the adrenal medulla of the rat. It appears that the expression and possible effects of orexins in the gut have just not been considered.

Orexin-immunoreactive neurons were found in both submucosal (Fig. 3A) and myenteric ganglia (Fig. 3B). Nevertheless, orexin antibodies marked distinct populations of enteric neurons that represent only a small subset (~25%) of the neurons that make up the ENS. Two neurochemically distinct, nonoverlapping populations of orexin neurons were identified in submucosal ganglia in the guinea pig ileum: noncholinergic neurons that contained vasoactive intestinal peptide (VIP), and cholinergic neurons that costored substance P.

VIP-ergic neurons are known to terminate near secretory epithelial cells, the function of which is to secrete chloride into the intestinal lumen (125). Chloride secretion, with accompanying fluid accumulation, is one of the ways the intestine lubricates itself, ensuring appropriate mixing and flow of digesta along its length. Substance P is a marker of submucosal primary afferent neurons that have been shown to project to the mucosa and to carry sensory information from the gut lumen to submucosal and myenteric ganglia (25). Intrinsic primary afferent neurons are critical for orchestrating the coordination of motility and secretion (125–127) and are essential for the initiation of peristaltic activity (25). Orexin-like immunoreactivity was also displayed by putative primary afferent neurons in the myenteric plexus. In addition, orexin neurons in both plexuses displayed leptin receptor immunoreactivity; therefore, like their CNS counterparts, orexin cells in the gut may be able to integrate this signal from adipose tissue, along with nutrient signals from the mucosa.

We had previously shown that leptin receptors are found in the ENS (27). Glucoreponsive enteric neurons, like pancreatic β-cells and glucoreponsive VMH neurons (128), displayed leptin receptor immunoreactivity, and leptin inhibited the activity of these cells by opening ATP-sensitive potassium channels (27). Therefore, the gut, like the endocrine pancreas and hypothalamus, contains cells that seem specialized in the integration of messages coming directly from extracellular nutrients (glucose) and indirectly from hormones (leptin) that signal the nutritional state of the organism.

To determine whether orexin synthesis in the gut was responsive to hunger, we examined the distribution of...
orexin-like immunoreactivity in the ENS after a 3-d fast (26). Strongly immunoreactive orexin-containing cell bodies, not seen in fed controls, were observed in submucosal ganglia of hungry guinea pigs. As a result, significantly more orexin-positive submucosal neurons were found in preparations from fasted animals.

Fasting also activated orexin neurons, as demonstrated by the induction of nuclear immunoreactivity to the phospho-Ser (133) form of the Ca\(^{2+}\)/cAMP response element binding protein in submucosal orexin-immunoreactive cells (26). These findings are consistent with parallel experiments in the LHA showing that hypothalamic orexins are stimulated by hunger (1, 13). The effects of fasting on orexin and orexin receptor mRNA expression in the ENS have not yet been determined; however, plasma orexin-A concentrations are increased during fasting in humans (129) and rats (R. Ouedraogo and A. L. Kirchgessner, unpublished observations). Interestingly, plasma leptin concentrations are concomitantly and significantly decreased during fasting. Thus, peripheral orexin-A and leptin concentrations inversely change during fasting and appear to be correlated with energy metabolism in humans. The source of peripheral orexins has not yet been determined.

As in the CNS, orexin nerve fibers are observed to be abundant in the gut (26). Orexin fibers are varicose in appearance and encircle subsets of ganglion neurons (Fig. 3, A and B). Orexin varicosities display synaptophysin immunoreactivity; therefore, orexin appears to be in nerve terminals making synaptic contact. Orexin fibers are found in both myenteric and submucosal ganglia, and in the circular muscle, where they contain VIP. VIP is a marker of inhibitory motor neurons in the myenteric plexus and mediates relax-
oration of the circular muscle (24, 25). The presence of orexin in VIP-ergic nerve fibers suggests that orexin might modulate intestinal motility.

An extensive network of orexin-containing fibers is also found in the mucosa where nutrients are absorbed from the lumen of the gut. Orexin fibers encircle mucosal crypts (which contain secretory epithelial cells) and extend within the lamina propria to the tips of the villi (which contain absorptive and endocrine cells). In the guinea pig, these fibers costore SP; therefore, at least a subset is likely to originate from submucosal primary afferent neurons.

The mucosa is also innervated by extrinsic primary afferent neurons with cell bodies in vagal and dorsal root ganglia (130). It is possible, therefore, that some of the orexin innervation of the mucosa originates from extrinsic sources. Orexin fibers in the NTS (15) and the dorsal horn of the spinal cord (49), central areas associated with sensory transmission, support this idea. In addition, Bingham et al. (41) demonstrated recently that both orexin-A and OX1R are present in dorsal root ganglion neurons, as well as the spinal cord, in the mouse. Furthermore, rat nodose ganglion neurons display orexin-A and OX1R immunoreactivity, and at least a subset of these cells innervates the stomach (131). Whether orexin-containing neurons in dorsal root ganglia innervate the gut will need to be examined in future studies. Nevertheless, these studies demonstrate that orexin and orexin receptors are found in both intrinsic and extrinsic primary afferent neurons and that sensory afferents are likely to be activated by orexins released from peripheral (enteric?) sources. Thus, orexins probably play a role in sensory transmission.

In parallel to the distribution of orexin-immunoreactive axons, orexin receptors are widely distributed in the ENS. We examined the distribution of orexin receptor protein and mRNA in the gut, by immunocytochemistry and RT-PCR, respectively (26). Orexin receptor-like immunoreactivity was found in close proximity to orexin-containing nerve fibers in the stomach and small intestine, OX1R was found in the circular muscle. Since OX1R expression was detected in total RNA isolated from strips of circular muscle, this finding suggests that smooth muscle cells may express OX1R. OX1R immunoreactivity was also displayed by submucosal and myenteric neurons, many of which were contacted by orexin varicosities. OX1R mRNA expression in enteric neurons was confirmed by RT-PCR. In addition, OX1R immunoreactivity was displayed by nerve fibers in ganglia, muscle, and mucosa, consistent with a presynaptic localization of the receptor and/or presence of receptor on sensory afferents.

Interestingly, OX2R immunoreactivity appeared to be localized to enteroendocrine cells in the intestinal mucosa. Thus, orexins might be able to modulate the release and/or effects of hormones via activation of OX2R. Support for this idea comes from a recent study, which showed that orexin potentiates the increase in $[Ca^{2+}]_i$ in response to CCK, in the intestinal mucosa of rats (132).

To determine whether orexin receptors were functionally active, we examined the response of submucosal neurons in the guinea pig ileum to orexin-A by intracellular electrophysiology (26). Orexin-A increased spike activity in these cells, similar to the increase in neuronal excitation observed in CNS neurons (42). In addition, orexin-A affected synaptic activity recorded in these cells. Bath application of the peptide reduced the amplitude of both stimulus-evoked fast and slow excitatory postsynaptic potentials and evoked slow inhibitory postsynaptic potentials. The fact that orexins directly increased the excitability of submucosal neurons and modulated both excitatory and inhibitory synaptic transmission, presumably by acting directly on axon terminals, suggests that the peptides could ultimately increase or decrease the activity of innervated enteric circuits.

C. Orexin-containing endocrine cells

In addition to enteric neurons, numerous endocrine cells in the stomach and throughout the intestine displayed orexin-like immunoreactivity (Fig. 3C and Ref. 26). The gut, therefore, may well be under the influence of orexins released from local neurons as well as orexins secreted as hormones from endocrine cells. The finding that both endocrine cells and neurons in the gut contained orexin was not surprising. CCK is released from enteroendocrine cells in the duodenum in response to a meal. It then stimulates the endings of vagal afferent neurons and results in reflex inhibition of gastric emptying and satiation (20–23). In addition, CCK is also found in the ENS where it acts as a neuromodulator and influences intestinal motility (24).

Orexin immunoreactivity colocalized with gastrin in the antrum of the stomach and OX1R was found on cells in the gastric corpus (26). Gastrin is a peptide hormone that was discovered in 1905 as an acid secretagogue (133). Gastrin is released into the circulation after a meal and stimulates gastric acid secretion through mobilization of histamine from enteroendocrine-like cells. Histamine, in turn, then stimulates the parietal cell to secrete acid (134). Thus, based on the localization of orexin and OX1R immunoreactivity in the stomach, it seems likely that orexin influences gastric acid secretion.

Recently it was shown that central administration of orexin-A stimulates gastric acid secretion in rats, and that this effect is mediated by central OX1R activation (106). Surprisingly, no effect on gastric acid secretion was seen during peripheral administration of the peptide.

In the intestinal mucosa, a subset of orexin-A-immunoreactive cells displays 5-hydroxytryptamine (5-HT) immunoreactivity (26). Based on this finding, orexin-immunoreactive endocrine cells were identified as enterochromaffin (EC) cells. Furthermore, EC cells expressed OX2R (135); therefore, orexins might be able to modulate 5-HT release and possibly regulate their own release in an autocrine-like fashion. 5-HT is released from EC cells in response to mucosal stimulation and activates both intrinsic (20, 24, 25) and extrinsic (136) 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 (137). It is not yet known whether orexin-A is released from EC cells; however, orexins, similar to 5-HT and several other gut hormones, can modulate gastrointestinal motility (26, 135, 138).
D. Orexins and motility

To investigate whether peripheral orexins specifically regulate motility, we examined the effects of orexin-A on the reflex-initiated rate of propulsion of an artificial fecal pellet in the guinea pig distal colon (26). Incubation of colonic segments with orexin-A caused a dose-dependent increase of the rate of propulsion. Thus, enteric orexin receptors are functional, and activation of these receptors can modulate motility. An excitatory action of orexin-A on muscle contraction has also been observed in human colon (138). In contrast, iv infusions of orexin-A inhibit the migrating motor complex in the small intestine of rats, inducing a more “fed-like” motility (135). The migrating motor complex is a specific motor pattern evoked by fasting that sweeps along the gut. Of particular interest is the fact that iv infusion of orexin-A produced a similar inhibitory effect as VIP on the migrating motor complex (135, 139). Since orexin nerve fibers in the circular muscle of the rat duodenum appear to contain VIP (135), the inhibitory effect of orexin-A on the migrating motor complex may be mediated by VIP or, at least, involve the same mechanism as VIP.

Convergent information suggests that orexins modulate motility through peripheral actions of the peptides. The fact that in both rat and mouse, orexin-A was not detected in the brain after iv administration, although high levels were found in the blood, further supports this idea (41). However, we still 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, orexin-A could modulate motility by acting on receptors located at nerve synapses within the enteric plexuses, mucosa, and/or muscle. In addition, since enteric cells of the mucosa express OX2R, the effects of orexin may be mediated through the EC cell, which contains possible motility-regulatory peptides.

VII. The Orexin System in the Pancreas

Endocrine cells in the pancreas also display orexin-like immunoreactivity (Fig. 3D and Ref. 26). Double-label immunocytochemistry revealed that orexin-A is present in insulin-immunoreactive cells of guinea pigs, rats, and mice (26). As constituents of the pancreatic β-cells, it is conceivable that orexins are released along with insulin and that they might function as hormones and/or paracrine or autocrine substances. Insulin-immunoreactive islet cells also display OX1R-like immunoreactivity, and OX1R mRNA was detected in the rat pancreas (26). Thus, orexins could potentially modulate insulin secretion. Recent studies support this idea. Nowak et al. (140) examined the effects of orexins on insulin release in rats and found that sc orexin-A stimulated insulin secretion in a dose-dependent manner (Fig. 4). In addition, orexin-A was also effective in stimulating insulin release in an in vitro perfusion system (140). Nevertheless, from these studies, it is not known whether orexin-A increases insulin release by acting directly on the β-cells.

Changes in insulin secretion produced by orexin-A may contribute to the increased food intake evoked by the peptide. Insulin will decrease circulating glucose levels and this could lead to feeding. In addition, insulin secretion occurs as part of the secretory response to cephalic stimulation (57, 141). Parallel to secretion, gastrointestinal motility is enhanced. Thus, the increase in secretion and motility produced by orexins suggests that the peptides might play a role in the integrative response of the gut and pancreas to cephalic stimulation.

Very recently, we have shown that orexin-A immunoreactivity is also displayed by the pancreatic α-cells (R. Ouedraogo and A. L. Kirchgessner, unpublished data). Low glucose stimulates the release of orexin-A from rat-isolated islets. Moreover, orexin-A evokes the release of glucagon. These findings indicate that, like neurons in the hypothalamus and gut, orexin-containing pancreatic α-cells are glucosensitive and become activated during periods of hypoglycemia. The stimulation of orexin release by low glucose might potentiate the effects of glucagon on glucose production during periods of hypoglycemia.

In addition to endocrine cells, orexin-like immunoreactivity is also found in nerve fibers in pancreatic ganglia, and pancreatic neurons display OX1R-like immunoreactivity (26). Ganglia are found throughout the pancreas, and pancreatic neurons contain most of the neuroactive substances found in enteric neurons (142). No neuronal cell bodies contain orexin; therefore, it is likely that the orexin nerve fibers originate from neurons located outside the pancreas. Pancreatic ganglia receive an extensive innervation from neurons located in the stomach and duodenum (115, 142). Enteropancreatic neurons reside in myenteric ganglia, which also contain orexin neurons; however, it is not known whether these cells project to the pancreas.

VIII. Other Homeostatic Systems Affected by the Orexins

The location of orexin-producing cells in the perifornical and LHA and their extensive projections in the brain and spinal cord suggested that the peptides might have roles in other homeostatic processes (3, 14, 15). Intracerebroventricular administration of the orexins has been shown to affect not only feeding but also several other systems. Orexins increase blood pressure and heart rate (143, 144), affect the
release of LH, GH, and PRL (145–147), increase drinking (148) and locomotor activity (149), and maintain wakefulness (15, 88, 89). Since orexin receptors are expressed in the adrenal medulla (124), orexins may also modulate epinephrine release. These findings indicate that orexins play a role in the regulation of the autonomic and neuroendocrine systems, including stimulation of sympathetic nerves (150). Interestingly, many of these effects are also associated with changes in food intake and gastrointestinal motility.

For example, sleep has been shown to be a major determinant of interdigestive (151, 152) and digestive (153–155) motility. Sleep is associated with diminished intestinal motility during the night and the day (154), suggesting that intestinal motility is regulated not only by circadian rhythm (156). Fasting alters sleep-wake cycles (151) and evokes a specific motor activity, the migrating motor complex, in the gut (152). Thus, sleep-wake, motility, and feeding patterns appear to be coordinated, and orexins may play a significant role in coordinating these complex physiological activities.

IX. Conclusions and Future Directions

In this article, I have summarized evidence for the existence of a brain-gut network of orexin-containing cells that appears to play a role in the acute regulation of energy homeostasis. We know that orexins are found in hypothalamic and enteric neurons that are activated by fasting (15, 26) and increase sensory arousal, wakefulness, food intake, gastrointestinal motility, and secretion (15, 26, 106). Hypothalamic orexin-containing neurons are sensitive to nutrient (glucose) and hormonal (leptin) messages (91, 92, 98, 104, 105) and are likely to be the glucosensitive neurons in the LHA that are activated when blood glucose falls (91, 99). Orexin neurons in the gut express leptin receptors (26), and, like the hypothalamus, the ENS contains both glucosensitive and glucoresponsive cells (36). Thus, it is likely that enteric orexin neurons are glucosensitive, although this interesting possibility remains to be tested in future studies. Nevertheless, these findings are consistent with the idea that glucosensitive cells in the brain and gut that are stimulated when extracellular glucose falls below a certain threshold might regulate the release of orexins. Such cells may be directly activated either through their glucose sensor or indirectly through the release of an inhibitory substance from neighboring glucoresponsive cells (128).

Vagal primary afferent neurons are sensitive to glucose (157), and we have found that orexin-A and orexin receptor immunoreactivity is displayed by vagal afferent neurons innervating the gut (131). Thus, orexins are present in the brain-gut axis and are likely to play a role in conveying sensory information from the gut to the brain. In support of this idea, orexins have been noted in nerve fibers/terminals in the NTS (3, 15); however, it has been assumed that innervation of the NTS originates entirely from orexin neurons in the LHA. The presence of orexin immunoreactivity in nodose ganglion cells (131) indicates that orexin innervation of the NTS also consists of vagal afferent terminals.

Hypothalamic orexin neurons receive sensory information from the gut, via an ascending projection from the NTS, and are able to modify vagal reflexes via a descending projection to the DMV. Signals that reach the NTS are initiated by mechanical or chemical stimulation of the gut during food ingestion, neural input related to energy metabolism in the liver, and humoral signals, such as CCK, that are released upon nutrient stimulation of endocrine cells lining the intestinal lumen. The presence of OX1R immunoreactivity within neuronal cell bodies in the nodose ganglion (131) suggests that orexin receptors are found on gut afferent fibers and that orexins can modulate primary afferent information from the gut to the brainstem. Orexin receptors are also found on nerve fibers in the lamina propria of the intestinal mucosa (26); therefore, orexins are likely to influence neural sensations conveyed by intrinsic primary afferent neurons (127).

Glucose-sensitive endocrine cells in the gut and pancreas express orexins and orexin receptors. Recent work demonstrating that luminal glucose induces the release of 5-HT from intestinal EC cells, and that 5-HT is responsible for eliciting the powerful vagal nodose neuronal responses evoked by glucose (157), suggests that EC cells might act as glucose sensors. Most gut endocrine cells have surfaces bearing microvilli that are directly apposed to the gut lumen. These surfaces are detectors that “taste” the luminal contents, in response to which the cells release hormones that enter the perfusing vasculature and/or excite afferent nerve endings beneath the mucosal epithelium (20). Orexins and OX2R are found in EC cells (26, 135); therefore, orexins might act as hormones and/or be able to modulate 5-HT release and possibly regulate their own release in an autocrine-like fashion.

Orexins are also found in gastrin-producing cells of the stomach (26). Gastrin cells are sensitive to glucose, since gastric release and gastric acid secretion are reduced during hyperglycemia (158). Interestingly, orexins are not found in gut cells that produce the incretin hormones, glucagon-like peptide 1 and glucose-dependent insulino tropic peptide, or the satiety hormone CCK. Since the incretins and CCK are released in response to a meal, it appears that orexins are selectively produced in cells that are responsive to acute reductions in energy substrate availability (glucose). The presence of orexin A in pancreatic α-cells and the stimulation of orexin release by low glucose in rat isolated islets (R. Ouedraogo and A. L. Kirchgessner, unpublished observations) further supports this hypothesis.

In spite of the recent effort that has been made to define the role of the orexins in the periphery, not much is known about the orexin-signaling system in the gut or pancreas. For example, we do not know the signals that trigger orexin release or whether the peptides act as hormones and/or neuromodulators, and we know very little about the outcome of the effects of orexins on gut motility or secretion.

Transgenic (orexin/ataxin-3) mice, depleted of orexin neurons have been generated, and these animals convincingly demonstrate that orexins influence feeding and energy homeostasis (19). The orexin/ataxin-3 mice eat less during the night when most feeding occurs and become obese even on a normal diet. If orexins are expressed in the gut, enteric neurons should also be ablated, causing gastrointestinal disorders. No defects have yet been reported in the ENS of...
Orexin/ataxin-3 mice. The absence of these reports, however, should not be interpreted to mean that the ENS of these animals is normal. Their ENS has not been investigated in any detail. The possibility also exists that the orexin promoter fragment used in the transgenic mice is not efficient in enteric neurons. Clearly, future experiments should determine whether orexin/ataxin-3 and/or orexin knockout mice have any defects in gut and/or pancreatic function, especially during conditions of hypoglycemia.

The role of orexins in sensory transmission also needs to be carefully examined. In addition to intrinsic and vagal primary afferent neurons, orexins and orexin receptors have recently been found in dorsal root ganglion cells (41). Moreover, peripheral orexins were shown to have analgesic properties (41). Thus, manipulation of spinal and/or enteric onexinergic systems may be efficacious in inflammatory conditions of the bowel in which hyperalgesia is a significant factor.

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