The other side of the orexins: endocrine and metabolic actions

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Taylor, Meghan M., and Willis K. Samson. The other side of the orexins: endocrine and metabolic actions. Am J Physiol Endocrinol Metab 284: E13–E17, 2003; 10.1152/ajpendo.00359.2002.—Although it is clear that the orexin/hypocretin peptides have a significant, physiologically relevant role in sleep/wakefulness, a broader picture has emerged indicating metabolic actions that may depend upon both neural and endocrine mechanisms for their manifestation. The ability of exogenous peptide to activate sympathetic tone, increase locomotor activity, and alter feeding behavior, together with the observed alterations in those functions in knockout animals, strongly suggests important neural actions of the endogenous orexins/hypocretins. Likewise, the action of exogenously administered peptides to alter endocrine function, in particular corticotropin release, has now been mirrored by potential endocrinopathies in knockout animals. Thus these pluripotent peptides hold great potential not only for the treatment of human narcolepsy but also to provide insight into the coordinated regulation of multiple physiological systems.

food intake; autonomic nervous system; narcolepsy; corticotropin; corticosterone

A REMARKABLE CONVERGENCE of independent efforts occurred early in 1998, when the La Jolla and Dallas groups simultaneously reported the predicted (6) or elucidated (29) structures of two peptides that would become named the hypocretins (6) or, alternatively, the orexins (29). These peptides, known to be processed from the same prohormone, were recognized to be produced in brain in a remarkably circumscribed location, the lateral hypothalamus/perifornical area, a region known to be important in feeding behavior (29). Axonal projections to multiple brain sites (4–6, 21, 25, 29, 38) predicted a diversity of biological actions, and it was clear from the beginning that the peptides exerted membrane effects similar to recognized neurotransmitters (6, 8, 42) and might have a significant role in the central nervous system (CNS) regulation of feeding behavior (13, 29, 31). For simplicity, these peptides will be referred to as the orexins. Orexin A (hypocretin-1) is a 33-amino acid peptide with two internal disulfide linkages, a pyroglutamyl residue in the NH2 terminus and an amidated COOH terminus. Orexin B (hypocretin-2) is 28 amino acids in length, lacks internal disulfide bonds, but similarly has an amidated COOH terminus. In their initial description of the orexins, Yanigisawa’s group (Sakurai et al., Ref. 29) also identified the two orexin receptors, OX1R and OX2R. Although both are G protein-coupled receptors, OX1R appears to couple via the Gq/11 subclass of heteromeric receptors, and OX2R may couple through either the Gq/11 or the Gi/o subclasses (29, 42). It is clear that OX1R prefers orexin A as a ligand, whereas OX2R binds orexin A or orexin B with similar affinities (29). Finally, it should be recognized that, in addition to a broad projection field originating from the orexin-producing neurons in the lateral hypothalamic/perifornical area, unique distributions of OX1R and OX2R sub-
types exist (17, 19, 29, 41), establishing the potential for the development of pharmacological agents that interact uniquely at a single subclass of orexin receptor and therefore act as selective agonists or antagonists for specific biological actions of the endogenous peptides.

**BIOLOGICAL ACTIONS OF THE OREXINS/HYPOCRETINS**

Although it is agreed that exogenously applied orexin can stimulate feeding and that orexin production is determined by the metabolic state of the animal (see review by Mieda and Yanagisawa, Ref. 20), most investigators recognize the effects of the orexins to be less potent than those of neuropeptide Y (NPY; see review by Smart et al., Ref. 37). However, it must be recognized that multiple peptide systems interact to control the feeding response, and the hierarchy within those systems is not clearly established. Pretreatment control the feeding response, and the hierarchy within those systems is not clearly established. Pretreatment with NPY antagonists significantly decreased the feeding response to exogenously administered orexin (7, 44), suggesting that NPY neurons are located downstream in the “wiring” diagram; however, evidence does exist for a potential opposite to be true, i.e., orexin neurons located farther downstream to NPY in a series circuit controlling feeding (22). There is strong evidence for the innervation of NPY neurons by orexin terminals in the arcuate nucleus of the hypothalamus (5, 11), and central administration of a dose of orexin that stimulated feeding significantly increased NPY mRNA in that nuclear group as well (16). Convincing evidence for a physiologically relevant role for the endogenous orexins in the CNS control of feeding comes from passive immunoneutralization studies, in which compromise of the bioactivity of endogenous orexin (22, 43) with neutralizing antibodies reduced feeding in response to an overnight fast. At least one group, with access to the compound, observed decreased feeding in response to exogenously applied orexin or fast-induced feeding after administration of the relatively selective nonpeptide OX1-R antagonist SB-334867 (27). Orexin knockout mice (preprohormone gene deletion) ate less than wild-type littermates (2), again suggesting a physiologically relevant role for endogenous orexin in the regulation of appetite. However, these mice grew normally; thus some other determinant of growth or body weight must compensate for the decreased feeding. There are two excellent possibilities: motor activity and metabolic state.

Early on, it was recognized that orexins stimulated motor activity and apparently increased arousal state (8, 30). The peptides also stimulate sympathetic nervous system activity (3, 30, 34, 36), and it was thought that the increase in feeding might actually have been secondary to the increased arousal state or metabolic demand (18). It is clear that locomotor activity and time spent in the awake state increased in the animals administered orexin (8). Thus, in addition to stimulating feeding, these peptides stimulated energy expenditure (45). Things became much clearer when Yanagisawa and colleagues (Chemelli et al., Ref. 2) once again demonstrated their technical expertise by developing an orexin gene knockout mouse. This model proved the importance of the endogenous orexin system to sleep/wakefulness but at the same time cast doubt on the physiological relevance of the observed effects of the peptides on feeding behavior and metabolism. These mice displayed a behavioral phenotype quite similar to human and canine models of narcolepsy (2, 15, 25), with distinct periods of behavioral arrest and the intrusion of rapid-eye-movement (REM) sleep into the normal waking state. If the primary function of the orexins were to control feeding and metabolic rate, as the pharmacological studies had suggested, then these animals would have been expected to display phenotypic changes reflective of the loss of those activities. However, the mice were reported to be otherwise phenotypically normal, and their general metabolic state, as mirrored by serum electrolytes and glucose levels, was similar to that of the wild-type littermates at 14–15 wk of age. In addition, the mice were fertile; thus the pharmacological effects previously reported on reproductive hormone (8, 26, 28, 39) and growth hormone (8) secretion did not appear to have a physiological expression in these animals at the times studied.

**DEVELOPMENT OF THE OREXIN NEURON “DROPOUT MODEL” REVEALS THE POTENTIAL PHYSIOLOGICAL RELEVANCE OF ENDOCRINE AND METABOLIC ACTION OF THE OREXINS/HYPOCRETINS**

Although the embryonic orexin gene knockout mice provided the key evidence that the pharmacological effects of orexin on sleep/wakefulness had potential physiological relevance and drew the attention of most investigators away from the endocrine and metabolic effects of the peptides, that model did not entirely recapitulate the pathology of human narcolepsy (2). On the contrary, it had been recognized that human narcolepsy was in almost all cases not a frank lack of orexin gene expression throughout life, but instead a gradual loss of orexin-producing neurons in the hypothalamus with aging (24, 40). Thus to faithfully recreate the mouse equivalent of the human model, Yanagisawa and colleagues (Hara et al., Ref. 9), in yet another brilliant series of experiments, created mice harboring the toxic ataxin-3 gene attached to the orexin promotor. The ataxin-3 gene encodes a protein with extended polyglutamine repeats that caused apoptosis when expressed in transfected cells in vitro (46). Already by 2 wk of age, the expression of the transgene could be detected in orexin-producing neurons, and by 4–8 wk of age, the majority of the orexin-producing neurons had been eliminated. Similarly, by 8 wk of age in the ataxin-3-transgenic mice, no preproorexin mRNA could be detected, whereas the expression of two other peptides known to be involved in the control of feeding, NPY and melanin-concentrating hormone, appeared normal (9).

By 6 wk of age, the orexin/ataxin-3 mice (9) were observed to display periods of behavioral arrest similar
to those previously reported by these investigators in the orexin-knockout animals (2). Fragmentation of sleep/wakefulness characteristic of the orexin-knockout animals was also observed in the orexin/ataxin-3 mice. This included increased frequency of entry into and duration of REM sleep. Thus these mice, because of the delayed onset of compromise of orexin-producing neurons, more faithfully mirrored the situation in human narcolepsy, which is characterized by a slowly progressive disease due to the “dropping out” of orexin neurons in the lateral hypothalamus (40).

The appearance of the alterations in sleep/wakefulness in these mice was predictable; however, additional observations made by this group of investigators were not, and these surprises provide important insight into the potential endocrine and metabolic actions of the endogenous orexins. One might have expected that, as these orexin/ataxin-3 mice aged, they would lose weight compared with wild-type controls due to the loss of the orexigenic action of the peptides. Instead, even with a predicted decrease in food intake, they became obese (9). The obese phenotype might have been due to the fact that the orexin/ataxin-3 mice displayed decreased spontaneous motor activity during the dark phase compared with controls, and thus their energy expenditure might possibly have been less, leading to obesity. This would suggest that the ability of exogenous peptide to stimulate arousal and sympathetic nervous system and locomotor activity (3, 8, 30, 36) in the earlier pharmacological studies had physiological correlates.

A most intriguing finding reported by Hara et al. (9) was that the orexin/ataxin-3 mice developed, along with obesity, what appeared to be late-onset diabetes (type 2 diabetes). Earlier work in the preproorexin gene knockout animals had not suggested any alterations in glucose homoeostasis, and there are no reports of direct effects of orexin on insulin secretion from isolated pancreatic islets or β-cells themselves. There is one report that intravenous administration of orexin elevates circulating insulin and glucose levels in rats, and that orexin A stimulates insulin release in vitro from isolated, perfused pancreas preparations (23), but then only at doses higher than those present in the gland or the circulation. The observed phenotype of obesity and diabetes in the orexin/ataxin-3 mice resembles the situation in some human narcoleptic patients (10, 35). It also mirrors the situation in cortisol excess, in which the obesity is the result of a combination of fat redistribution, due to altered insulin sensitivity, and increased appetite. It is our hypothesis that the orexin/ataxin-3 mice represent exactly the same pathology.

Central administration of orexin in conscious rats resulted in a behavioral response similar to the stress response (8, 30). This included increased grooming and motor activity (e.g., searching behavior). These injections also resulted in increased sympathetic nervous system tone, reflected by increased heart rate and blood pressure (3, 30, 36). It is not known whether the orexin/ataxin-3 mice display a “reset” or depressed autonomic system, and it will be important to examine that possibility. Similarly, central administration of orexin stimulated corticotropin (ACTH) and corticosterone release (8, 12, 14, 34) in vivo, an action that was blocked by pretreatment of the animals with a corticotropin-releasing factor (CRF) antagonist (12, 34). CRF is a recognized anorexigenic/anxiogenic peptide (8), and the loss of orexin neurons might predict not only a decrease in CRF release into the hypothalmo-pituitary portal vessels in response to stress but also the loss of a potential stimulatory action of orexin on CRF neurons important in the “shut-off” of feeding cues. Thus, in the absence of orexin, less CRF would be released under basal and stimulated conditions, and the animal, in addition to feeding for longer periods, would become corticosterone deficient. Instead, quite the opposite was observed. The animals ate less and appeared to experience corticosterone excess. The decreased feeding observed clearly might be due to the loss of the orexigenic drive provided by the endogenous peptide, but what might explain the apparent corticosterone excess?

We have thought that the ability of centrally administered orexin to stimulate ACTH and corticosterone release in vivo (8, 12, 14, 34) was a reflection of a generalized stress effect of the exogenous peptide (30, 36) and not an indication of the role of endogenous orexin in the day-to-day control of the hypothalamo-pituitary-adrenal axis. Instead, we have identified direct anterior pituitary effects of the orexins/hypocretins (32) that potentially explain the metabolic phenotype of the orexin/ataxin-3 mice (9). Orexin-immunopositive nerve terminals exist in the external lamina of the median eminence (4), the site of diffusion of neuroendocrine factors into the hypophysial-portal vessels connecting the hypothalamus with the anterior lobe of the pituitary gland. In addition, both of the OX1R and OX2R subtypes exist in the anterior lobe, and the OX1R has been localized to corticotrophs in the human gland (1). We have demonstrated the significant inhibitory effect of the orexins on CRF-stimulated ACTH release from cultured, anterior pituitary cells (32). The effect appeared to be mediated by the OX1R subtype and a PKC-dependent mechanism, suggesting activation of a Gq-initiated signaling pathway (32). More recently, we have demonstrated that orexin stimulated transient elevations in intracellular inositol trisphosphate levels (33), and Ferguson and Samson (A. V. Ferguson and W. K. Samson, unpublished observations) have observed calcium transients in fura 2-labeled, CRF-responsive pituitary cells in vitro (A. V. Ferguson and W. K. Samson, unpublished observations). This would suggest that binding of orexin to the OX1R on corticotrophs results in activation of phospholipase C, with subsequent formation of inositol trisphosphate and diacylglycerol (DAG). Because we have blocked the inhibitory action of orexin on CRF-induced ACTH release with the PKC inhibitor calphostin C, we hypothesize that the formation of DAG results in the activation of a potassium channel, resulting in hyperpolarization of the corticotroph and a “right shift” in the CRF concentration-response curve.
This hypothesis is supported by our demonstration that pretreatment of the cells with a concentration of the potassium channel inhibitor glyburide, which by itself did not alter basal or CRF-stimulated hormone secretion, was followed into adulthood, a clearer picture of the physiological relevance of the pharmacological effects of exogenously administered orexin began to appear. These mice are fertile and apparently grew normally, with the exception that they had decreased activity and food intake, in addition to the alterations in sleep/wakefulness. Thus previously described effects of exogenous peptide on reproductive hormone and growth hormone secretion were not reflected in these knockout mice (2). Metabolic alterations were recognized, however. When animals expressing the orexin/ataxin-3 gene product were followed into adulthood, a clearer picture of the physiological relevance of the pharmacological effects of exogenously administered orexin began to appear. These mice are narcoleptic and display decreased motor activity (9). In addition, they are obese and diabetic. The model will certainly provide additional insight into systems and metabolism. However, observations from the study of the preproorexin gene knockout mice suggested that several of the observed endocrine actions of pharmacologically administered orexin were in, all likelihood, without physiological correlate. Those mice were fertile and apparently grew normally, with the exception that they had decreased activity and food intake, in addition to the alterations in sleep/wakefulness. Thus previously described effects of exogenous peptide on reproductive hormone and growth hormone secretion were not reflected in these knockout mice (2). Metabolic alterations were recognized, however. When animals expressing the orexin/ataxin-3 gene product were followed into adulthood, a clearer picture of the physiological relevance of the pharmacological effects of exogenously administered orexin began to appear. These mice are narcoleptic and display decreased motor activity (9). In addition, they are obese and diabetic. The model will certainly provide additional insight into the control of sleep/wakefulness, but even more importantly, it may provide information on the genesis of some forms of insulin-resistant diabetes, in particular those associated with excess corticotroph production, e.g., Cushing’s disease. This may also stimulate clinical researchers to examine the possibility that a subset of human narcoleptic individuals are cushingoid. Finally, these mice will provide a model in which to examine the unique, concordant actions of the peptides on feeding and energy utilization, which in the case of orexin, unlike those of other orexigenic peptides (9, 18), are positive for both food intake and energy expenditure.

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