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Neuropeptides controlling energy balance: orexins and neuromedins

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Abstract

In this section we review the feeding and energy expenditure effects of orexin (also known as hypocretin) and neuromedin. Orexins are multifunctional neuropeptides that affect energy balance by participating in regulation of appetite, arousal, and spontaneous physical activity. Central orexin signaling for all functions originates in the lateral hypothalamus–perifornical area, and is likely functionally differentiated based on site of action and on interacting neural influences. The effect of orexin on feeding is likely related to arousal in some ways, but is nonetheless a separate neural process that depends on interactions with other feeding related neuropeptides. In a pattern distinct from other neuropeptides, orexin stimulates both feeding and energy expenditure. Orexin increases in energy expenditure are mainly by increasing spontaneous physical activity, and this energy expenditure effect is more potent than the effect on feeding. Global orexin manipulations, such as in transgenic models, produce energy balance changes consistent with a dominant energy expenditure effect of orexin. Neuromedins are gut-brain peptides that reduce appetite. There are gut sources of neuromedin, but likely the key appetite related neuromedin producing neurons are in hypothalamus and parallel other key anorectic neuropeptide expression in the arcuate to paraventricular hypothalamic projection. As with other hypothalamic feeding related peptides, hindbrain sites are likely also important sources and targets of neuromedin anorectic action. Neuromedin increases physical activity in addition to reducing appetite, thus producing a consistent negative energy balance effect. Together with the various other neuro-peptides, -transmitters, -modulators and -hormones, neuromedin and orexin act in the appetite network to produce changes in food intake and energy expenditure, which ultimately influences the regulation of body weight.

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Brain orexins and energy balance

Orexin

When the discovery of a novel peptide apparently limited to cell bodies in the hypothalamus was announced in 1998 (1, 2), interest was high due to the possibility of its involvement with feeding. The peptide, dubbed orexin by Sakurai et. al. and hypocretin by de Lecea et. al., was independently discovered in two laboratories using very different methods (1, 2). One group isolated the long form of orexin, orexin A (OXA), by searching for ligands for “orphaned” G-protein coupled receptors (2). The second group first isolated the precursor protein, preproorexin, in 1996 using a subtractive PCR technique to recover hypothalamus-specific proteins (3) but did not publish a detailed investigation of the precursor or its derivatives until early in 1998 (1).

The initial reports of these discoveries showed that the orexins are a family containing two peptides, the 33 amino acid OXA (hypocretin-1) and the shorter 28 amino acid orexin B (OXB, hypocretin-2), both derived from the precursor protein, preproorexin (PPO), through proteolytic processing (1, 2). The PPO gene, which is highly conserved across species, has some similarities with the secretin/incretin family of peptides (1), and appears to have arisen early during chordate evolution through a circular mutation of an incretin gene (4). Orexin has been identified in all major vertebrate taxa, including fish (5, 6), amphibians (7-9), reptiles (10), birds (11), and mammals (12). Within the central nervous system, preproorexin mRNA was initially reported to be limited to cell bodies in the lateral hypothalamus (LH) (3). While there is some evidence for orexin neurons in other brain regions, including the paraventricular hypothalamic and supraoptic nuclei, amygdala, median eminence, and ependyma (13-16), to date there is no conclusive evidence of orexin mRNA in any brain region except the lateral hypothalamus.

The orexins bind to two G-protein coupled receptors; OXA binds equally to either orexin receptor 1 (OX₁R) or orexin receptor 2 (OX₂R); OXB binds to both receptors but displays moderate selectivity for OX₂R (2, 17). Orexin A and B have been shown to increase the postsynaptic activity of GABAergic and glutamatergic cells (18). The orexins may also affect the presynaptic effect of Ca²⁺-dependent transmitters by increasing calcium levels, both through mobilization of internal Ca²⁺ stores and through secondary influx of external calcium (17).

Although the total number of orexin neurons is fairly small, axonal projections from these cells extend from the LH to many regions of the rat brain and spinal cord (13, 16, 19-22), and the distribution of these neurons and axonal projections is very similar across rodent strains and species (16). The overall distribution of orexin fibers in the brain and spinal cord allows this small population of neurons to play roles in integrating multiple autonomic and behavioral functions, primarily feeding, sleep/wake behavior, and arousal (23-35), but also including nociception, respiratory, motor, neuroendocrine and cardiovascular systems (16, 19-22, 36-41). Disruptions or deficiencies in orexin signaling have been linked to a number of sleep/wake and endocrine disorders in humans and in animal models (33, 42-45).

There is also strong evidence for an important role for orexin outside of the central nervous system. Both orexin and orexin receptors are present in peripheral tissues. Both PPO and orexin receptor mRNA are present in the gut in several species, including rats, guinea pigs, dogs, horse, deer, mice, sheep, and humans (46-51); however at least one report questions these findings (52). Additionally, PPO mRNA has been identified in the heart and testicular tissue of rats (53), and orexin receptors have been found in rat lung, the adrenal glands and gonads of both rats and sheep, and in the enteric nervous system of several species (48, 49, 53, 54).

Orexin and feeding

The hypothalamic distribution of cell bodies containing the precursor protein suggested that orexins are involved in feeding behavior. Prior to the discovery of orexin, the only other peptide known to be found in cell bodies limited to the LH was melanin concentrating hormone (MCH), a peptide known to be involved the regulation of feeding (55). Evidence that 48 hours of fasting elicited a 2.4-fold increase in rat preproorexin mRNA (2) quickly prompted more extensive investigation into the relationship between the orexins and feeding. Early experiments showed that injections of OXA and OXB elicit ingestion in rats, although the effects of OXA appeared to be stronger than those of OXB, perhaps due to its more stable structure (2). This difference in the orexigenic effects of OXB in comparison to OXA has been replicated several times, with most studies suggesting that OXB is less effective than OXA in eliciting feeding or drinking behavior (2, 26, 56). In some cases OXB has been ineffective in eliciting any ingestive behavior whatsoever (57, 58).

Orexin effects on ingestive behavior appear to depend upon interactions with other food-related signaling systems, such as neuropeptide Y (NPY), leptin, MCH, ghrelin, galanin, and agouti-related protein (47, 59-64). For example, the ingestive behavior stimulated by orexin is attenuated or blocked by leptin, a potent inhibitor of food intake (reviewed in 61). In one study, pretreatment with leptin blocked orexin-induced activity in nearly half of the orexin responsive neurons identified in the arcuate nucleus (Arc) (64). In addition, leptin injections in the rat are capable of blocking both OXA-induced feeding behavior as well as NPY-induced Fos immunoreactivity in OXA cells (23). Leptin may block the effects of orexins directly or indirectly, as some OXB cells have been shown to express leptin receptors, and NPY cells in the rat and monkey Arc receiving orexin fiber contact also express leptin receptors (60). Orexin appears to have a reciprocal blocking effect on leptin, as OXA administered intravenously reduces plasma leptin concentrations in humans (47).

Previous studies have shown that orexin and arcuate nucleus NPY neurons have reciprocal functional connections important in feeding (29, 60, 65). Orexin neurons in the LH send projections to the Arc, and these fibers form synaptic contacts with NPY-containing neurons in this nucleus (60). Administration of orexin increases expression of the early-active gene cFos in Arc NPY neurons (29). In turn, Arc NPY neurons project to the LH where they make synaptic contact with orexin neurons (59, 60, 65). While a large number of these NPY-orexin contacts in the LH appear to originate in the Arc, NPY neurons in other regions are also known to project to the LH and may contribute to this NPYergic input to orexin neurons (65). Injection of NPY into the perifornical LH (PeF), in which orexin neurons are

found, robustly stimulates food intake (66), and this induction of intake shows a circadian pattern of effectiveness, eliciting the greatest response during the active period (67), matching the endogenous circadian patterns of cFos expression in orexin neurons (68). Orexin neurons in the PeF are known to express NPY Y4 receptors, and cFos expression is increased in orexin neurons following application of NPY or a Y4-specific agonist (23, 69). While central orexin injection increases food intake, the effect is at least partly dependent on activation of NPY neurons, as orexin-induced intake is attenuated (but not blocked) by administration of an NPY Y1 receptor antagonist (29); this effect is complicated by the finding that NPY tonically pre- and post-synaptically inhibits orexin neurons via a Y1-specific pathway (70). Interestingly, NPY-induced food intake appears to be partly dependent on orexin, as treatment with an orexin antibody reduces (but does not eliminate) NPY-induced food intake (23), and anatomical evidence suggests orexin neurons may be a downstream target of NPY action in feeding (59). Both orexin and NPY neurons express receptors for the hunger-signaling hormone leptin (60), suggesting that while NPY neurons might represent the main target of this hormone, orexin neurons are also responsive to peripheral signals of energy balance (71).

There are several lines of evidence that suggest interactions between orexin and blood glucose levels. Insulin-induced hypoglycemia results in a rapid rise in nuclear cFos expression in OXA cells of the rat (72). Orexin-containing pancreatic islet cells also contain insulin in humans, and some of these cells express orexin receptors (47). Intravenous orexin administration raises insulin levels in the blood, presumably by stimulating pancreatic cells expressing such receptors (47). In addition, there are some indications that defects in the orexin system may affect the regulation of glucose in humans. For example, in humans with narcolepsy, a condition associated with low or nonexistent levels of orexins (45, 73), there appears to be a higher risk of non-insulin dependent diabetes (74).

Despite the documented relationship between the orexins and feeding and satiety systems, there is some controversy over the actual effect orexins have on feeding behavior. The administration of orexins into the central nervous system has not always reliably increased feeding behavior (reviewed in 75). Some have argued that increased ingestion following orexin administration is due solely to the increased locomotor activity caused by orexin; however at least one study suggests that locomotor and feeding effects of orexin are independent rather than coupled (24). While it is generally agreed that the orexins are not as potent a stimulator of feeding as NPY, for example, the relative strength or weakness of the orexins as compared to other peptides such as MCH has not been clearly established (56, 58). Indeed, in studies performed in various laboratories, the orexins elicited an ingestive response ranging between very robust (2, 29), moderate (56, 57), or weak (58).

The differences in feeding behavior elicited in individual studies may be explained by several factors. First, orexins have been shown to increase both GABA and glutamate release in the rat in vitro (18). These peptides thus appear to have the ability to affect the fast synaptic excitatory or inhibitory activity of many parts of the hypothalamus. Therefore, the reported effects of centrally administered orexins may not be physiologically relevant, as spillover into other brain regions could activate or inhibit systems not normally involved in the feeding effects of orexin. The actual discrete local effects of naturally released peptides

are presumably much more finely controlled by the brain than even the most carefully placed injection. Indirect actions or spillover effects of injected orexins have been proposed as explanations for differences seen in several studies (56, 58). Secondly, the relative degree of feeding behavior observed after introduction of orexin may be related to stress, as at least some orexin-induced ingestive responses rely upon interactions between orexin, NPY, and corticosterone levels (29, 60, 76, 77). Finally, orexin-induced feeding might be time-dependent. Circadian responsiveness of the feeding effect of OXA in the rat has been observed in at least one study, with an increase in food intake following OXA injection only occurring during the light phase of the cycle (24).

Although the exact role of the orexins in feeding has yet to be established, it is possible that the orexins are involved in the coordination of locomotor activity and arousal in response to stress and variation in food availability. During short-term food deprivation, orexin receptor mRNA is upregulated (78). Orexins promote wakefulness (34, 79, 80), reliably increase locomotor activity in rats (24, 81, 82), and occasionally lead to increased searching and exploratory behavior (83, 84). A decrease in food availability may thus increase arousal at times that the animal is normally quiescent, leading to increased locomotion and searching behaviors. By modifying the timing of arousal, the orexin system might increase the chance of the animal encountering a food source that is not available at other times of the day. The orexin system is clearly uniquely situated for involvement in the coordination of an interrelated suite of behaviors related to food intake and arousal.

Orexin and arousal

The overall distribution of orexin fibers in the brain has suggested that the orexins play a role in a number of systems, including the maintenance of arousal (80, 85). Orexin fibers have been shown to project to various brain nuclei implicated in the control of sleep state (20-22, 86, 87). Application of OXA in the locus coeruleus (80, 88) and lateral preoptic area (79) of the rat have been shown to increase wakefulness, primarily through a decrease in rapid eye movement (REM) sleep (88). Activity in locus coeruleus neurons increases following application of OXA (80, 85, 88). In contrast to OXA, OXB does not seem to affect wakefulness (88).

The orexin cells also receive input from brain systems involved in regulation of sleep-wakefulness. In mammals, circadian organization of activity including sleep-wake behavior is regulated by the endogenous clock located in the suprachiasmatic nucleus (SCN) (reviewed in 89). Orexin cell bodies receive both limited direct contact from the SCN (90), as well as substantial indirect contact from the SCN via the medial preoptic area (91). Introduction of chemicals known to increase arousal in rats, such as methamphetamines or the anti-narcoleptic drug Modafanil, increase nuclear Fos expression in orexin cell bodies (89, 92, 93). Furthermore, increasing the behavioral arousal of rats by sleep deprivation induced due to handling also increases the expression of nuclear Fos in OXA cells (93). The orexins thus appear to be capable of both receiving information relating to the arousal state of the animal, and relaying arousal information to other nuclei known to promote wakefulness. The finding that a defect in the orexin system is associated with the sleep

disorder narcolepsy (33, 92, 94, 95) has strengthened the association between the orexins and arousal.

Orexin actions on endocrine and autonomic systems

Orexin may also be involved in the regulation of autonomic functions. There are extensive projections from orexin neurons to hindbrain nuclei that regulate cardiovascular and sympathetic processes (20, 96). Several studies have shown that application of OXA increases heart rate, blood pressure, and respiration rate in rats and mice (39, 40, 96-98). Body temperature, which generally rises during active periods and decreases when animals are quiescent, increases following injection of OXA (99), but not after injection of OXB (84). The increase in body temperature following application of OXA does not appear to be a result of increased locomotor activity (99).

Finally, orexins have been implicated in modulation of the hypothalamic-pituitary-gonadal (HPG) axis at several levels. First, within the hypothalamus, orexin has been shown to stimulate the release of gonadotropin releasing hormone (GnRH) (100). Cells containing GnRH receive direct contact from orexin fibers in rats and sheep (101, 102), and in rats GnRH neurons have also been shown to express orexin receptors (101). In addition, orexin projections to the hypothalamic magnocellular nuclei that project to the pituitary also appear to be important in HPG regulation. Magnocellular neurons in the Pa express orexin receptors, and these receptors are selectively upregulated during the estrous cycle and early lactation in rats (103). At the level of the pituitary, more evidence for orexin involvement in HPG regulation has been found. Specifically, both rat and human pituitary express orexin receptors (53, 104), and, OXA acting on these receptors appears to directly block GnRH-mediated release of luteinizing hormone in proestrous female rats (100). With respect to the gonads, testicular tissue in rats express orexin, and both rat and sheep testicular tissue expresses orexin receptor mRNA (53, 54). Although orexin receptors have been found in rat ovary, unlike in male gonads, orexin mRNA appears to be absent (53). Although the specific actions of orexin on gonadal tissue are currently unknown, the presence of orexin and orexin receptors in the gonads suggests the possibility that orexins may affect the HPG axis at all three levels.

Orexin, physical activity and energy expenditure

Orexin augmentation of energy expenditure was reported shortly after initial reports describing the orexins in the literature (1, 2, 58). Orexin A infusion into the third ventricle increased metabolic rate, and the increase was more robust in the dark cycle (active phase) relative to the light cycle (resting phase) in mice (58). In contrast, equimolar doses of OXB were ineffective. The circadian variation in OXA-induced metabolic rate (58) parallels nuclear c-Fos immunoreactivity (an indicator of cellular activity) in orexin neurons across the light/dark cycle (93), which highlights the contribution of orexins to basal metabolism. The stimulatory effect of ventricular OXA in mice (105) was confirmed and was later extended to rats as OXA stimulated oxygen consumption normalized to body weight (106, 107). Orexin augmentation of whole body energy expenditure can be attributed to specific brain sites of action. Orexin A infusion into the arcuate nucleus increases oxygen

consumption in anesthetized rats (108) and increases thermogenesis after infusion into the hypothalamic paraventricular nucleus (PVH) and rostral lateral hypothalamus (rLH) in conscious rats (82, 109-111). In contrast, OXA has no effect on oxygen consumption in anesthetized rats after infusion into the locus coeruleus (LC), paraventricular thalamic nucleus (PVT), caudal lateral hypothalamus (cLH), PVH, medial preoptic area (MPO), and the dorsomedial and ventromedial hypothalamic nuclei (108).

Inconsistent effects of OXA infusion in the PVH and LH are likely due to differences in the anesthesia state, dose range of OXA tested, location of the injectate (rLH vs. cLH) and the endpoint reported between studies. That OXA reduces respiratory quotient (58) underscores the importance of measuring the change in both oxygen and carbon dioxide during indirect calorimetry experiments and reporting energy expenditure as heat production. Finally, the opposing effect of OXA in the rLH and cLH on energy expenditure demonstrates that behavioral effects of neuropeptides can be regionally specific similar to the feeding effects of OXA in the lateral hypothalamus (112), urocortin in the lateral septum (113) or inhibition of NPY-induced feeding by naltrexone in the nucleus of the solitary tract (NTS) (114).

Physical activity

Physical exertion requires ATP utilization and substrate oxidation, and as such physical activity ranging from muscle movements during volitional motion to actions as small as postural maintenance incur an energetic cost (115). The effects of orexin on physical activity, including increases in locomotion, rearing, grooming, and burrowing activities, require muscular contraction and thus expend energy. Consistent with this idea, acute intra-PVH OXA dose-dependently increases physical activity and energy expenditure in rodents (81, 82) and chronic OXA increases physical activity and reduces body weight (116).

Injections of OXA and, to a lesser extent OXB have been shown to increase locomotor activity (24, 81, 82, 84, 99, 117, 118) and burrowing behavior (83). Activation of orexin neurons appears to be positively correlated to the level of locomotor activity in several rodent species (93, 119, 120). Orexin B does not appear to have as strong an effect on general locomotor activity as does OXA, but has been shown to be more effective than OXA in eliciting searching behavior (83, 84) or exploration of novel environments (84). Importantly, the increase in locomotor activity observed after application of OXA does not appear to be related to the concurrent increase in feeding often observed following orexin injections (24), suggesting that feeding and activity effects of orexin may be influenced by different neural mechanisms. Face washing and grooming behavior also increase in frequency following injections of OXA but not OXB (83, 84, 117, 118, 121). The increase in grooming following injection of OXA in the rat is blocked by prior application of a CRF antagonist, suggesting that the behavior may be linked to stress (117). Both the grooming and locomotor effects of orexins may also involve interactions with dopaminergic (118) and serotonergic (118, 121) systems.

Orexin A stimulates several types of physical activity following ventricular (80, 83, 96, 99, 117, 118, 122-126) or peripheral infusion (127). Multiple sites of action appear to underlie this stimulatory effect. Indeed, OXA infusion into the LH (24, 81, 128), PVH (81, 82, 109, 110), substantia nigra (81), tuberomammillary nucleus (TMN), LC and dorsal raphe (129)

stimulates physical activity. To date orexin A has had a stimulatory effect on locomotion after site-specific infusion with one exception. Relatively high doses of OXA (3 μ g) in the PVT inhibited locomotion (130). Unlike locomotion, effects of OXA on other types of physical activity appear to be site-dependent. España et al (124) compared the effect of OXA in the lateral ventricle and into forebrain nuclei on grooming, rearing, and quadrant entries. While there was no effect of OXA in the substantia innominata on the behaviors tested, OXA in the lateral ventricle and intra-MPO increased all behaviors, while OXA in the medial septum stimulated grooming only. Finally, it is important to note that measurement duration should be considered when comparing efficacy of OXA within the same site. For example, in one study OXA in the nucleus accumbens shell (AcbS) increased locomotor activity during the 30 to 120 minute post-injection interval (131), but there was no effect of OXA 10 to 30 minutes post-injection, consistent with others who reported no effect of OXA during the 0 to 30 minute post-injection time interval (132). This lack of effect in the immediate post-injection period may be due to heightened physical activity due to handling during the injection process, as increased physical activity for up to 20 minutes post-injection in all treatment groups has been shown (24).

Orexin stimulation of locomotor activity may rely in part on projections to the thalamic intergeniculate leaflet (IGL). The IGL is a thin structure located in the lateral geniculate complex of the thalamus, between the ventral and dorsolateral geniculate nuclei (VG and DLG, respectively) (133, 134). NPY neurons in the IGL project directly to the SCN (135). Patterns of cellular activation of IGL NPY neurons are correlated with activity patterns in rodents (136-138). Manipulations which mimic release of NPY into the SCN results in changes in patterns of physical activity in rodents, and perturbations of the IGL or of NPY cells therein block these changes in activity (139-143). Orexin neurons send moderately dense axonal projections to the IGL, but little or no fibers to the VG or DLG; the presence of these fibers is highly consistent between species (16, 19-21, 87, 144). Data suggests that at least some of the mechanisms through which orexin affects physical activity might rely on these projections to the IGL. Orexin fibers form close appositions with NPY neurons in the IGL and appear to form functional contacts with these cells (120, 145). Patterns of cFos activation in orexin neurons are correlated with cFos expression patterns in NPY neurons of the IGL (138, 145), suggesting that orexin neurons influence the role of IGL NPY neurons in control of behavioral activity. Furthermore, in one study, orexin fibers in the IGL preferentially approached NPY cells, which expressed cFos in patterns correlating with physical activity (145), suggesting that cellular activation of these neurons is influenced by orexigenic input.

Consistent with effect of orexins on physical activity, orexin augments muscle tone, which would be expected to influence energy expenditure. Orexin A infusion into the LC (146) or the alpha gigantocellular reticular nucleus in the medioventral medullary region (147) increases hindlimb muscle tone. Likewise, OXA infusion into the trigeminal motor nucleus and hypoglossal motor nucleus increases EMG activity, an indicator of muscle activity, in the masseter and geniosglossus muscles (148). In contrast, OXA microinjection into the pontine inhibitory area (146) or the ventral gigantocellular reticular nucleus (147) inhibit hindlimb muscle tone.

Orexin effects on sympathetic outflow - cardiovascular and thermoregulatory systems

Orexin effects on cardiovascular and thermoregulatory systems have been extensively reviewed (39, 149-153). Brain areas classically involved in thermoregulation and cardiovascular function receive orexin projections and express orexin receptors (20, 154-156), providing a neuroanatomical basis for orexin involvement in cardiovascular function and thermoregulation. Behavioral studies further suggest that sympathetic outflow is orexin-mediated. Orexin A infusion into the lateral ventricle increases renal sympathetic nerve activity (157, 158), plasma epinephrine (157), noradrenaline release (159), and firing rate of sympathetic nerves innervating the interscapular brown adipose tissue (iBAT) (160-162), which would be expected to increase thermogenesis. Likewise, intrathecal OXA infusion (163) and in vitro application (156) stimulate sympathetic outflow. Tachycardia is observed following OXA in the lateral (106, 157, 160-162) and fourth ventricle (96), or following infusion intracisternally (164) and intrathecally (163) but not intravenously (164). Moreover, the pressor response to orexin is similar to the effect of orexin on heart rate (38, 157, 158, 163, 164). These sympathetic and cardiovascular effects are clearly mediated by multiple sites of action. Orexin A infusion into the rostral lateral ventral medulla (RVLM) (164), NTS (165, 166), Arc (108), PVH (160, 167), and the diagonal band of Broca induce tachycardia (160), and OXA in the RVLM (164) and NTS (165, 166) stimulate mean arterial pressure. In contrast, infusion into the nucleus ambiguus (98) or the sub-fornical organ induces bradycardia (168). While OXA in the nucleus ambiguus (98) has no effect on blood pressure, intra-subfornical organ OXA (168) reduces blood pressure, thereby indicating site-specific actions of orexin on cardiovascular responses. In contrast to the cardiovascular response to orexin, orexin action on temperature appears to be consistently observed independent of route of administration. Orexin A increases colonic (162), iBAT (161, 162), cutaneous (161), and core body temperature (96) following infusion into the lateral and fourth ventricle (96), Arc (108), and the diagonal band of Broca (169). A recent report showed that chronic infusion of the OX1R antagonist (SB-334867-A) into the lateral ventricle increased iBAT temperature during the dark cycle and UCP1 protein expression in the iBAT (170). Furthermore, there is no effect of acute OXA on colonic temperature (80) or of chronic OXA infusion on iBAT temperature (171). It is plausible that these discrepancies may be due to differences in dose or duration of injectate, site of administration, location of thermistor, or duration of measurement. Together, these data indicate orexin action is sympathoexcitatory, which would supplement energy expenditure due to orexin-mediated metabolic rate and physical activity.

Orexin integration of feeding and physical activity

A greater understanding of how orexin may integrate information important to both energy balance and physical activity may be derived from studying the effects of gains in orexin action, and loss of orexin function studies. Loss of orexin neurons, either by lesion, genetics or post-natal ablation, affects feeding behavior and physical activity. Likewise, orexin neurons respond to changes in energy balance brought about by nutritional status and exercise, suggesting that orexins receive information relevant to both behaviors. While there

is not enough existing information to understand how orexin may integrate feeding and activity, orexin neuron circuitry would lend itself well to such a role: orexin neurons receive input from several important energy sending areas, and project mono- and multi-synaptically throughout the brain to multiple brain areas with diverse functionality. A physiological state of energy deficit would confer interoceptive cues signaling appetitive drive, whereas states of energy excess could signal for energy loss, perhaps via non-exercise energy expenditure. Orexin neurons project to and excite arcuate neuropeptide Y neurons (172). During food restriction this signal is robustly enhanced, demonstrating that negative energy states are sensed by orexin neurons, which respond by enhancing orexigenic tone to restore energy balance. Whether this relationship exists for physical activity is unknown. Exercise may induce an interoceptive state of temporary satiety, which is not perceived as a situation of negative energy balance by the brain. Yet, existing data suggest that exercise may also stimulate orexin neurons (120, 173). While orexin neurons are glucose sensing, and thus could respond to glucose alterations associated with physical activity, significance at the synapse level is unclear. Exercise-associated motor activity or food anticipatory-associated activity could also be two mechanisms responsible for this induction of orexin activity. The metabolic and sensory milieu following exercise vs. spontaneous physical activity is likely very different, but to date there are no studies differentiating between these activity states and the corresponding effects on orexin signaling. As mentioned above, the induction of spontaneous physical activity by orexin A is inconsistent with an endocrine feedback loop, in which one might expect a reduction of orexin activity after motor activity. While currently there is no explanation for this relationship between orexin A and physical activity, it is likely that identification of subpopulations of orexin neurons and clarification of their functional roles will shed light on orexin and physical activity interactions.

Orexin effects: energetic balance

As indicated above, orexin elevates both eating behavior and energy expenditure. The increase in both of these outputs does not fit the typical profile for neuromodulators of energy balance (174), which is that of an inverse relationship between the two outputs of feeding and energy expenditure; e.g. if a compound increases food intake, it concurrently reduces energy expenditure, whereas if a compound reduces food intake, it increases energy expenditure. These opposing outputs have been noted for most described neuromodulators of food intake and energy expenditure (174). Why this is the case is unclear, but this model is attractive, as it fits a classic homeostatic model of regulation, and allows for the categorization of compounds (neuropeptides, -modulators, -transmitters and -hormones) into either the “satiety” or “obesigenic” category. In what category lies a compound that elevates both food intake and energy expenditure? This lack of ability to classify orexin as either obesity producing (via enhanced food intake) or obesity preventive (via enhanced energy expenditure) has created a confused discussion of this peptide’s function. The purpose of this section is to integrate the knowledge of orexin effects on food intake and energy expenditure, and clarify the role that orexin has in body weight regulation.

As suggested by multiple studies, the orexin signal in different brain areas with different functionality translates to different outcomes; orexin in one area may have a feeding effect, whereas in another area, an effect on physical activity and energy expenditure. The sum total

of all these actions will influence body weight. We clearly do not have the type of comprehensive evidence that is needed to understand precisely what effect endogenously produced orexin, acting in all projection sites, has on eating behavior and energy expenditure in different physiological states, but we can start to make some assumptions about this based on receptor profiles, site functionality, pharmacological studies and by studying obesity prone and resistant models.

Narcolepsy in humans is accompanied by significantly reduced or absent orexin, and significantly increased body mass (44, 73, 175), suggesting that the overall effect of orexin is obesity resistance. Animal models of orexin-loss support this observation, as mice with transgenic ablation of orexin neurons become obese (95). Orexin global over-expression has mixed results on body weight, but this can be expected in studies in which the orexin signal is placed in areas in which it is not normally expressed. While there are few studies of orexin overexpression exclusively in the LH area, at least one study suggests that increasing orexin expression protects against weight gain in animals fed on a high-fat diet (176).

Clearly, orexin action is determined not just by orexin output, but also by receptor expression. The consequences of increased orexin receptor expression have only just begun to be studied. Work from our laboratory shows that obesity resistant rats have increased physical activity and that resistance to obesity is associated with increased orexin receptor expression (24, 81, 128). Obesity resistant rat activity is also more sensitive to orexin (24, 81, 128) (81, 82, 109, 110), suggesting either elevated capacity for orexin action via increased receptor. Additionally, early levels of physical activity are associated with lifelong reduced adiposity in obesity resistant rats (Teske, in press). These findings suggest that the lean phenotype of OR rats may be explained by their high level of physical activity, which appears to be mediated by orexin receptors. This finding is supported by work showing that enhanced orexin receptor expression in rats mitigates the propensity for obesity on a high-fat diet (176).

Thus, the energetic consequence of these two behavioral outputs, when added up on a caloric basis result in negative energy balance, and reduces body weight. As orexin enhances feeding behavior and physical activity in a site-specific manner, it is unclear at this point where the dominant effects on each output is taking place. Nonetheless, the calories taken in by the effects of the orexin signal are outweighed by those expended via physical activity. Based on this, orexins may be considered as potential targets for obesity therapy, rather than obesigenic.

Neuromedins

Neuromedins are one group of gut-brain peptides that illustrate how the gut and brain communicate and act in parallel to modulate energy balance. Though less is known about neuromedins compared to other gut-brain peptides such as CCK or ghrelin, interest in the role of neuromedins in energy balance has increased markedly over the past few years. Of the neuromedins, neuromedin U (NMU) is perhaps the subject of most investigation (177). Related peptides, including neuromedins B, C, K, L, N, and S, likely serve similar or related functions, and some exert their actions through the same receptors. Investigations into these

peptides have revealed that, like other gut-brain hormones, a variety of physiological functions and behaviors are influenced by neuromedins. The peripheral actions of neuromedins have been reviewed (177-180); here, we will focus primarily on central actions of the neuromedins, particularly of neuromedin-containing neurons found in the brain.

Neuromedin U was first identified as a spinal cord peptide that induced smooth muscle contractions in the uterus, the tissue for which it is named (181, 182). NMU is found at high levels within the intestine, specifically in neurons of the enteric nervous system (177, 183, 184). Whether or not circulating NMU from peripheral sources acts on the brain to exert its actions is not known. Like other gut-brain peptides, there are sources of neuromedins intrinsic to the brain (185), possibly reflecting parallel gut/brain systems seen in other gut-brain peptides. Within the brain, most attention has been given to NMU-containing cells in hypothalamic regions important in energy balance, particularly its actions in the PVH and Arc, specifically pro-opiomelanocortin (POMC)-containing arcuate neurons (186), and also dorsomedial hypothalamus (184, 186, 187). Several non-hypothalamic regions also contain NMU-immunoreactive neurons and fibers, however, including hindbrain regions important in arousal and energy balance that are also responsive to CCK (185, 188). Though reports vary somewhat in the pattern of NMU cell or projection distribution, this could be secondary to cross-reactivity with other neuromedins, such as neuromedins S (NMS), which share the same receptors (189, 190).

As would be expected from the cell and fiber distribution, NMU receptors or their mRNAs have been identified in hypothalamic brain regions associated with energy balance. Two receptor subtypes have been identified in the brain, NMR1 and NMR2, with the reports citing NMR2 as the most prevalent receptor in the central nervous system and NMR1 found primarily in peripheral tissues (191, 192). Within the hypothalamus, NMR2 have been identified in the PVH, dorsomedial nucleus, the dorsal periventricular nuclei, surrounding the ventromedial nucleus, and in the ependymal layer of the ventricle; NMR2 are also found in the brainstem (186, 188, 193, 194). Other brain systems also contain neuromedins binding or NMR2, including the hippocampus (188, 194-196). NMR1 has also been identified in brain regions, including the amygdala (197). Functional studies have used Fos to identify brain regions and systems that are activated by central (i.c.v.) treatment with NMU; these include the most common hypothalamic nuclei associated with NMU—PVH, Arc, dorsomedial nucleus, and lateral hypothalamic area—but also forebrain regions associated with motivation and reward (amygdala, nucleus accumbens, frontal cortex), the supraoptic nucleus, and the hindbrain parabrachial nucleus and nucleus of the solitary tract (197-199). Though the hypothalamic regions and, to a lesser extent, the hindbrain regions, have received attention in functional behavioral or physiological studies (188, 200-206), relatively little attention is paid to the potential functions of neuromedins in the forebrain.

Neuromedins are one set of neuropeptides that act on the “anorexigenic” arm regulating appetite (107, 193, 198, 203). Activation of neuromedin receptors decreases food intake and increases energy expenditure and physical activity (107, 190, 200, 201, 203, 207). Moreover, commonalities in the behavioral and energetic actions of neuromedins and homologous peptides can be seen in non-mammals, even invertebrates (208-214). It has been hypothesized that one action of leptin is to stimulate the release of NMU, through

which it exerts its actions on metabolism (186, 187, 203, 215). Transgenic over expression of NMU in mice leads to hypophagia and leanness (216), and deletion of the gene for NMU results in obesity, hyperphagia, and decreased physical activity (217). Some have found that mice lacking the NMUR2 gene do not show this phenotype (218), but others have found that NMUR2-deficient mice are lean, hypophagic, and somewhat resistant to weight and fat gain on a high-fat diet (190). In humans, variants in the gene encoding pro-NMU are associated with obesity (219). This led to interest in neuromedins as a potential target for weight-loss therapy. Though acute NMU leads to decreased food intake and increased energy expenditure and physical activity, twice-daily intra-PVH treatment with NMU failed to induce significant weight loss (204). In contrast, chronic central (i.c.v.) infusions of NMU using osmotic minipumps significantly suppressed energy intake, body weight, and adiposity, (190) though this effect may depend on the diet (220).

The central actions of neuromedins are similar to several other neuropeptides commonly termed “anorexigenic,” such as corticotrophin-releasing hormone (CRH): decreased appetite, increased energy expenditure, and increased physical activity (201, 221, 222). In fact, neuromedins are likely to be one important component that activates brain CRH to affect behavior (202, 203, 223, 224); CRH also appears to be necessary for some of the behavioral effects of NMU (224). This may be one reason why neuromedins affect behaviors traditionally associated with brain CRH and, more generally, with the stress response (such as locomotion and increased grooming) (222, 223, 225). In fact, brain neuromedins affect several other functions and behaviors besides food intake, including reproduction, the sleep-wake cycle (226), hippocampal and memory function (196, 227), sympathetic outflow (228), reproductive and stress axis function (198, 204, 217, 225, 229-231), prolactin secretion (197), pain sensitivity (218), brain oxytocin and vasopressin systems (199, 206, 232, 233), and bone remodeling (234).

Orexins and neuromedins in the appetite network

Orexin and the neuromedins are multifunctional neuropeptides that participate in a wide variety of neuroregulatory processes. Each of these processes, including appetite, arousal and spontaneous activity, is the result of the combined output of many neuropeptides acting in a number of brain sites, all organized into a network. Thus there is a network of brain sites and activities for appetite, a related but distinct network for arousal, and another for physical activity. The actions of orexins throughout the brain are a particularly good illustration of this concept because orexins clearly perform different functions at different brain sites, even though the origin of orexin neurons is in a highly focused place in the lateral hypothalamus and perifornical areas. Work by Thorpe and Kotz (235) has shown that while there are certain brain sites, like rostral LH and paraventricular hypothalamus, where orexin increases feeding and also increases spontaneous physical activity, there are other brain sites like ventral tegmental area where orexin only affects feeding or locus coeruleus where orexin only affects activity.

Brain sites where orexin affects appetite are an important subset of the known sites involved in the appetite regulatory network. Orexin’s role at these brain sites ultimately results in increases in feeding, but the exact mode of producing this behavioral phenotype with respect

to neuronal function, and particularly the context of that neuronal function involving basal state and other neuromodulators is incompletely defined. Considering only the effect of orexin stimulation on the behavioral phenotype of appetite, it is important to note that orexin effects do not fit into a clearly established unidirectional action pathway. The example that establishes this concept is the interaction between orexin action in the lateral hypothalamus and neuropeptide Y action in the arcuate and paraventricular nuclei. As reviewed above, orexin in the LH can activate neuropeptide Y producing neurons in the arcuate nucleus. Neuropeptide Y action in the paraventricular nucleus can also activate orexin neurons in the lateral hypothalamus. Thus a bidirectional stimulatory pathway involving these two orexigenic stimuli can be identified. Further, both lateral hypothalamus and paraventricular nucleus are connected through other pathways involving other neurotransmitters with other components of the appetite regulatory network. In the case of orexins itself, feeding stimulatory signals from the LH project to rostral LH, paraventricular hypothalamus, and nucleus accumbens.

A linear action pathway cannot account for the known database of brain sites and signals that participate in appetite/body weight regulation, whose action and function rely upon inputs from each other and from peripheral signals. Bidirectional information transfer, as with the example of orexin and neuropeptide Y, is a common theme in this distributed network. There are many such examples of neural interactions, and these interactions indicate that no one 'regulator' is operating alone or within one brain area to determine the food intake behavioral response, but rather, a dynamic neural network of neurotransmitters at several brain sites are communicating with each other to determine this output. Therefore, the orexin effect on appetite is not a linear model with one initiation point, but a multipoint model. Further, there is cross talk between the networks for appetite, arousal and activity (among others), such that each of the networks responsiveness to orexin can be seen both as a function within that domain as well as contributing to the phenotypic output of other domains as well.

Orexin and neuromedin receptors as therapeutic targets

The pharmacologic efficacy of selective and dual orexin receptors antagonists has been tested. Scammell and Winrow recently reviewed the preclinical and clinical pharmacology of multiple orexin receptor antagonists and described their favorable therapeutic efficacy for insomnia (236). Despite this, the efficacy for other pathologies such as obesity remains to be determined. The lack of a commercially available orexin 2 receptor antagonist and comprehensive testing of antagonists on non-sleep related parameters that influence energy balance has hampered progress. The relevance of such testing is imperative as orexin stimulates energy intake, energy expenditure and promotes stabilization of the sleep/wake cycle, which together influence body weight regulation. Therefore, comprehensive testing in addition to distinguishing the functional specificity of the orexin receptors is necessary as it is unclear whether stimulation of one or both receptors is necessary and/or sufficient to elicit a behavioral response and thus a given pharmacologic effect. Based on the behavioral effects of orexin receptor antagonists (reviewed below), it appears that an orexin-based obesity treatment must promote satiety, stimulate energy expenditure and stabilize sleep/wake parameters.

Orexin 1 receptor antagonists

The first commercially available selective orexin 1 receptor antagonist, SB-334867, has been shown to reverse orexin A-induced feeding (27), physical activity-induced thermogenesis (82), grooming (121), sympathetic activity (237), arousal (238) as well as the delay in the normal transition from eating to resting (behavioral satiety sequence) induced by orexin A (239). Selective blockade of orexin 1 receptors by SB-334867 also attenuated orexin B-stimulated physical activity (84). In contrast, SB-408124 had no effect on sleep, physical activity, or body temperature after peripheral administration (240). Other selective orexin 1 receptor antagonists including SB-410220 (241) and diaryl urea analogues of SB-334867 (242) have been described pharmacologically; however, behavioral effects have yet to be determined.

Orexin 2 receptor antagonists

Two proprietary selective orexin 2 receptor antagonists have been reported. N-ethyl-2-[(6-methoxy-pyridin-3-yl)-(toluene-2-sulphonyl)-amino]-N-pyridin-3-ylmethylacetamide (EMPA) reduced dark cycle basal physical activity and [Ala¹¹, D-Leu¹⁵] orexin B-stimulated physical activity (243). Peripheral infusion of JNJ-10397049 promoted sleep and reduced basal physical activity and body temperature in the light and dark cycle (240).

Dual receptor antagonists

Actelion described the first dual orexin receptor antagonist as being most effective during the active phase of the light/dark cycle in rats, dogs and humans (244). Almorexant (ACT-078573) orally was shown to promote sleep and reduce home cage activity despite no effect on body temperature during the active dark period in rats. Parallel effects were observed in dogs with efficacious sleep promotion and physical activity reduction effects observed during the day but absent when Almorexant was administered prior to sleep. In humans, oral administration in the morning reduced clinical and subjective alertness and promoted sleep demonstrated by reduced latency with no adverse effects. Recently, Li et al reported that Almorexant reduced oxygen consumption and promoted sleep in rats after oral gavage in the dark cycle only and had no effect on body temperature (245). Additional biocomparison, tolerability, pharmacokinetic, and pharmacodynamic tests in humans suggest that Almorexant may be promising for treatment of insomnia (246, 247).

Several dual receptor antagonists developed by Winrow and colleagues at Merck have been described. Suvorexant (MK-4305) has been shown to reduce physical activity and promote sleep in rats, dogs and rhesus monkeys (248). Like its predecessor (249), DORA-1 promoted sleep, reduced basal physical activity and reduced physical activity stimulated by [Ala¹¹, D-Leu¹⁵] orexin B and amphetamine (250). In a similar manner, another dual orexin receptor antagonist based on a 1,4-diazepane central scaffold reduced basal dark cycle physical activity (251). From these studies, DORA-5 was developed and was shown to reduce home cage physical activity and increase sleep after oral administration (251). The clinical efficacy of another dual receptor antagonist, SB-674042, has also been demonstrated for insomnia (Reviewed in 236).

Some progress has also been made to exploit the brain neuromedin system for potential pharmacological treatment for obesity. An antagonist, R-PSOP, has been described (252). This antagonist binds competitively to the NMUR2 with high affinity, and significantly attenuates the nociceptive response induced by NMU-23 treatment. However, there have yet to be reports on the development of agents that might act to stem obesity by targeting central NM receptors to alter behavior or energy expenditure.

Summary

Orexin affects energy balance in several ways, notably by increasing feeding in some behavioral contexts and more potently by stimulating energy expenditure mainly through increasing the level of spontaneous activity. The brain site in which the orexin engages its receptor principally determines the regulatory functions of orexin, but taken together orexin mainly exerts negative energy balance influence. The two forms of orexin and the two receptor subtypes likely also play a role in differentiating function, as do the contexts of the receiving neuron and the network in which the signaling is taking place.

Neuromedin is an anorexic peptide with expression for the peptide and receptor in gut, hypothalamus and brainstem. There is evidence that neuromedin may play a major role in signaling certain kinds of satiety signals, such as leptin based signals. Neuromedin also contributes to negative energy balance influences by increasing physical activity and thereby energy expenditure.

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