Neuropeptides Controlling Energy Balance: Orexins and Neuromedins

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Abstract In this chapter, 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 the 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 other various neuropeptides, neurotransmitters, neuromodulators, and neurohormones, 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.

Keywords Brain • Feeding • Obesity • Physical activity

1 Brain Orexins and Energy Balance

1.1 Orexin

When the discovery of a novel peptide apparently limited to cell bodies in the hypothalamus was announced in 1998 (de Lecea et al. 1998; Sakurai et al. 1998), 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 (de Lecea et al. 1998; Sakurai et al. 1998). One group isolated the long form of orexin, orexin A (OXA), by searching for ligands for "orphaned" G protein–coupled receptors (Sakurai et al. 1998). The second group first isolated the precursor protein, prepro-orexin, in 1996 using a subtractive PCR technique to recover hypothalamus-specific proteins (Gautvik et al. 1996) but did not publish a detailed investigation of the precursor or its derivatives until early in 1998 (de Lecea et al. 1998).

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, prepro-orexin (PPO), through proteolytic processing (de Lecea et al. 1998; Sakurai et al. 1998). The PPO gene, which is highly conserved across species, has some similarities with the secretin/incretin family of peptides (de Lecea et al. 1998) and appears to have arisen early during chordate evolution through a circular mutation of an incretin gene (Alvarez and Sutcliffe 2002). Orexin has been identified in all major vertebrate taxa, including fish (Huesa et al. 2005; Kaslin et al. 2004), amphibians (Shibahara et al. 1999; Yamamoto et al. 2004; Singletary et al. 2005), reptiles (Farrell et al. 2003), birds (Ohkubo et al. 2002), and mammals (Sakurai et al. 1999). Within the central nervous system, prepro-orexin mRNA was initially reported to be limited to cell bodies in the lateral hypothalamus (LH) (Gautvik et al. 1996). While there is some evidence for orexin neurons in other brain regions, including the paraventricular hypothalamic and supraoptic nuclei, amygdala, median eminence, and ependyma (Chen et al. 1999; Ciriello et al. 2003a; Kummer et al. 2001; Nixon and Smale 2007), 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 (OX_1R) or orexin receptor 2 (OX_2R); OXB binds to both receptors but displays moderate selectivity for OX_2R (Sakurai et al. 1998; Smart et al. 1999). Orexin A and B have been shown to increase the postsynaptic activity of GABAergic and glutamatergic cells (van den Pol et al. 1998). The orexins may also affect the presynaptic effect of Ca^{2+}-dependent transmitters by increasing calcium levels, both through mobilization of internal Ca^{2+} stores and through secondary influx of external calcium (Smart et al. 1999).

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 (Chen et al. 1999; Nixon and Smale 2007; Cutler et al. 1999; Date et al. 1999;

Peyron et al. 1998; Nambu et al. 1999), and the distribution of these neurons and axonal projections is very similar across rodent strains and species (Nixon and Smale 2007). 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 (Niimi et al. 2001a; Kotz et al. 2002; Rodgers et al. 2000; Kunii et al. 1999; Haynes et al. 2000; Mondal et al. 1999; Yamanaka et al. 2000; Tsujino and Sakurai 2009; Nunez et al. 2009; Siegel 1999; Lin et al. 1999; Piper et al. 2000; Hungs and Mignot 2001), as well as nociception, respiratory, motor, neuroendocrine, and cardiovascular systems (Nixon and Smale 2007; Cutler et al. 1999; Date et al. 1999; Peyron et al. 1998; Nambu et al. 1999; Volgin et al. 2002; Zhang and Luo 2002; Samson et al. 1999; Shirasaka et al. 2002; Zhang et al. 2005a; Berthoud et al. 2005). Disruptions or deficiencies in orexin signaling have been linked to a number of sleep/wake and endocrine disorders in humans and in animal models (Lin et al. 1999; Petersén et al. 2005; Nevsimalova et al. 2005; Thannickal et al. 2000; Nishino et al. 2000).

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 (Kirchgessner and Liu 1999; Ehrstrom et al. 2005; Dall'aglio et al. 2009, 2008, 2011; de Miguel and Burrell 2002); however, at least one report questions these findings (Baumann et al. 2008). Additionally, PPO mRNA has been identified in the heart and testicular tissue of rats (Johren et al. 2001), and orexin receptors have been found in rat lung, in the adrenal glands and gonads of both rats and sheep, and in the enteric nervous system of several species (Dall'aglio et al. 2009, 2008; Johren et al. 2001; Zhang et al. 2005b).

2 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 in the regulation of feeding (Qu et al. 1996). Evidence that 48 h of fasting elicited a 2.4-fold increase in rat prepro-orexin mRNA (Sakurai et al. 1998) 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 (Sakurai et al. 1998). 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 (Sakurai et al. 1998; Kunii et al. 1999; Edwards et al. 1999).

In some cases, OXB has been ineffective in eliciting any ingestive behavior whatsoever (Sweet et al. 1999; Lubkin and Stricker-Krongrad 1998).

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 (Ehrstrom et al. 2005; Broberger et al. 1998; Horvath et al. 1999a; Schwartz et al. 2000; Takenoya et al. 2005; Sakurai 2003; Rauch et al. 2000). For example, the ingestive behavior stimulated by orexin is attenuated or blocked by leptin, a potent inhibitor of food intake (reviewed in Schwartz et al. 2000). In one study, pretreatment with leptin blocked orexin-induced activity in nearly half of the orexin-responsive neurons identified in the arcuate nucleus (Arc) (Rauch et al. 2000). 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 (Niimi et al. 2001a). 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 (Horvath et al. 1999a). Orexin appears to have a reciprocal blocking effect on leptin, as OXA administered intravenously reduces plasma leptin concentrations in humans (Ehrstrom et al. 2005).

Previous studies have shown that orexin and arcuate nucleus NPY neurons have reciprocal functional connections important in feeding (Yamanaka et al. 2000; Horvath et al. 1999a; Elias et al. 1998). Orexin neurons in the LH send projections to the Arc, and these fibers form synaptic contacts with NPY-containing neurons in this nucleus (Horvath et al. 1999a). Administration of orexin increases expression of the early-active gene cFos in Arc NPY neurons (Yamanaka et al. 2000). In turn, Arc NPY neurons project to the LH where they make synaptic contact with orexin neurons (Broberger et al. 1998; Horvath et al. 1999a; Elias et al. 1998). 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 (Elias et al. 1998). Injection of NPY into the perifornical LH (PeF), in which orexin neurons are found, robustly stimulates food intake (Stanley et al. 1993), and this induction of intake shows a circadian pattern of effectiveness, eliciting the greatest response during the active period (Stanley and Thomas 1993), matching the endogenous circadian patterns of cFos expression in orexin neurons (Martinez et al. 2002). 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 (Niimi et al. 2001a; Campbell et al. 2003a). 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 (Yamanaka et al. 2000); this effect is complicated by the finding that NPY tonically pre- and postsynaptically inhibits orexin neurons via a Y1-specific pathway (Fu et al. 2004). 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 (Niimi et al. 2001a), and anatomical evidence suggests orexin neurons may be a downstream target of NPY action in

feeding (Broberger et al. 1998). Both orexin and NPY neurons express receptors for the hunger-signaling hormone leptin (Horvath et al. 1999a), suggesting that while NPY neurons might represent the main target of this hormone, orexin neurons are also responsive to peripheral signals of energy balance (Meister 2000).

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 (Moriguchi et al. 1999). Orexin-containing pancreatic islet cells also contain insulin in humans, and some of these cells express orexin receptors (Ehrstrom et al. 2005). Intravenous orexin administration raises insulin levels in the blood, presumably by stimulating pancreatic cells expressing such receptors (Ehrstrom et al. 2005). 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 (Nishino et al. 2000, 2001), there appears to be a higher risk of non-insulin-dependent diabetes (Honda et al. 1986).

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 (Sutcliffe and de Lecea 2000)). 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 (Kotz et al. 2002). While it is generally agreed that the orexins are not as potent as NPY in being a stimulator of feeding, for example, the relative strength or weakness of the orexins as compared to other peptides such as MCH has not been clearly established (Edwards et al. 1999; Lubkin and Stricker-Krongrad 1998). Indeed, in studies performed in various laboratories, the orexins elicited an ingestive response ranging from very robust (Sakurai et al. 1998; Yamanaka et al. 2000), moderate (Edwards et al. 1999; Sweet et al. 1999), to weak (Lubkin and Stricker-Krongrad 1998).

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 (van den Pol et al. 1998). 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 (Edwards et al. 1999; Lubkin and Stricker-Krongrad 1998). Second, 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

(Yamanaka et al. 2000; Horvath et al. 1999a; Ida et al. 2000a; Jászberényi et al. 2000). 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 (Kotz et al. 2002).

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 (Lu et al. 2000). Orexins promote wakefulness (Piper et al. 2000; Methippara et al. 2000; Hagan et al. 1999), reliably increase locomotor activity in rats (Kotz et al. 2002, 2006; Kiwaki et al. 2004), and occasionally lead to increased searching and exploratory behavior (Ida et al. 1999; Jones et al. 2001). 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.

3 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 (Hagan et al. 1999; Horvath et al. 1999b). Orexin fibers have been shown to project to various brain nuclei implicated in the control of sleep state (Date et al. 1999; Peyron et al. 1998; Nambu et al. 1999; Moore et al. 2001; Mintz et al. 2001). Application of OXA in the locus coeruleus (Hagan et al. 1999; Bourgin et al. 2000) and lateral preoptic area (Methippara et al. 2000) of the rat has been shown to increase wakefulness, primarily through a decrease in rapid eye movement (REM) sleep (Bourgin et al. 2000). Activity in locus coeruleus neurons increases following application of OXA (Hagan et al. 1999; Horvath et al. 1999b; Bourgin et al. 2000). In contrast to OXA, OXB does not seem to affect wakefulness (Bourgin et al. 2000).

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 Weaver 1998). Orexin cell bodies receive both limited direct contact from the SCN (Abrahamson et al. 2001) as well as substantial indirect contact from the SCN via the medial preoptic area (Deurveilher and Semba 2005). Introduction of chemicals known to increase arousal in rats, such as methamphetamines or the antinarcoleptic drug modafinil, increases nuclear Fos expression in orexin cell bodies (Weaver 1998; Chemelli et al. 1999; Estabrooke et al. 2001). Furthermore,

increasing the behavioral arousal of rats by sleep deprivation induced due to handling also increases the expression of nuclear Fos in OXA cells (Estabrooke et al. 2001). 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 (Lin et al. 1999; Chemelli et al. 1999; Hungs et al. 2001; Hara et al. 2001) has strengthened the association between the orexins and arousal.

4 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 (Date et al. 1999; Zheng et al. 2005). Several studies have shown that application of OXA increases heart rate, blood pressure, and respiration rate in rats and mice (Shirasaka et al. 2002; Zhang et al. 2005a, 2005; Ciriello et al. 2003b; de Oliveira and Ciriello 2003). Body temperature, which generally rises during active periods and decreases when animals are quiescent, increases following injection of OXA (Yoshimichi et al. 2001), but not after injection of OXB (Jones et al. 2001). The increase in body temperature following application of OXA does not appear to be a result of increased locomotor activity (Yoshimichi et al. 2001).

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) (Russell et al. 2001). Cells containing GnRH receive direct contact from orexin fibers in rats and sheep (Campbell et al. 2003b; Iqbal et al. 2001), and in rats, GnRH neurons have also been shown to express orexin receptors (Campbell et al. 2003b). 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 (Wang et al. 2003a). At the level of the pituitary, much evidence for orexin involvement in HPG regulation has been found. Specifically, both rat and human pituitaries express orexin receptors (Johren et al. 2001; Blanco et al. 2001), and OXA acting on these receptors appears to directly block GnRH-mediated release of luteinizing hormone in proestrous female rats (Russell et al. 2001). With respect to the gonads, testicular tissue in rats expresses orexin, and both rat and sheep testicular tissues express orexin receptor mRNA (Johren et al. 2001; Zhang et al. 2005b). Although orexin receptors have been found in rat ovary, unlike in male gonads, orexin mRNA appears to be absent (Johren et al. 2001). 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.

5 Orexin, Physical Activity, and Energy Expenditure

Orexin augmentation of energy expenditure was reported shortly after initial reports describing the orexins in the literature (de Lecea et al. 1998; Sakurai et al. 1998; Lubkin and Stricker-Krongrad 1998). 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 (Lubkin and Stricker-Krongrad 1998). In contrast, equimolar doses of OXB were ineffective. The circadian variation in OXA-induced metabolic rate (Lubkin and Stricker-Krongrad 1998) parallels nuclear cFos immunoreactivity (an indicator of cellular activity) in orexin neurons across the light/dark cycle (Estabrooke et al. 2001), which highlights the contribution of orexins to basal metabolism. The stimulatory effect of ventricular OXA in mice (Asakawa et al. 2002) was confirmed and was later extended to rats as OXA-stimulated oxygen consumption normalized to body weight (Wang et al. 2001; Semjonous et al. 2009). 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 (Wang et al. 2003b) and increases thermogenesis after infusion into the hypothalamic paraventricular nucleus (PVH) and rostral lateral hypothalamus (rLH) in conscious rats (Kiwaki et al. 2004; Novak et al. 2006a, 2010; Teske et al. 2010). 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 (Wang et al. 2003b).

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 (Lubkin and Stricker-Krongrad 1998) 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 (Thorpe et al. 2003), urocortin in the lateral septum (Wang and Kotz 2002), or inhibition of NPY-induced feeding by naltrexone in the nucleus of the solitary tract (NTS) (Kotz et al. 2000).

6 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 incurs an energetic cost (Webb et al. 1980). 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 (Kotz et al. 2006; Kiwaki et al. 2004), and chronic OXA increases physical activity and reduces body weight (Novak and Levine 2009).

Injections of OXA and, to a lesser extent OXB, have been shown to increase locomotor activity (Kotz et al. 2002, 2006; Kiwaki et al. 2004; Jones et al. 2001; Yoshimichi et al. 2001; Ida et al. 2000b; Nakamura et al. 2000) and burrowing behavior (Ida et al. 1999). Activation of orexin neurons appears to be positively correlated to the level of locomotor activity in several rodent species (Estabrooke et al. 2001; España et al. 2003; Nixon and Smale 2004). Orexin B does not appear to have as strong effect on general locomotor activity as does OXA but has been shown to be more effective than OXA in eliciting searching behavior (Ida et al. 1999; Jones et al. 2001) or exploration of novel environments (Jones et al. 2001). 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 (Kotz et al. 2002), 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 (Ida et al. 1999, 2000b; Jones et al. 2001; Nakamura et al. 2000; Duxon et al. 2001). 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 (Ida et al. 2000b). Both the grooming and locomotor effects of orexins may also involve interactions with dopaminergic (Nakamura et al. 2000) and serotonergic (Nakamura et al. 2000; Duxon et al. 2001) systems.

Orexin A stimulates several types of physical activity following ventricular (Hagan et al. 1999; Ida et al. 1999, 2000b; Zheng et al. 2005; Yoshimichi et al. 2001; Nakamura et al. 2000; Matsuzaki et al. 2002; Sunter et al. 2001; España et al. 2001; Volkoff and Peter 2000; Samson et al. 2010) or peripheral infusion (John et al. 2000). Multiple sites of action appear to underlie this stimulatory effect. Indeed, OXA infusion into the LH (Kotz et al. 2002, 2006; Teske et al. 2006), PVH (Kotz et al. 2006; Kiwaki et al. 2004, 2006a; Novak et al. 2010), substantia nigra (Kotz et al. 2006), tuberomammillary nucleus (TMN), LC, and dorsal raphe (Kotz et al. 2008) 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 μg) in the PVT inhibited locomotion (Li et al. 2009). Unlike locomotion, effects of OXA on other types of physical activity appear to be site dependent. España et al. (2001) 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-min postinjection interval (Thorpe and Kotz 2005), but there was no effect of OXA 10–30 min postinjection, consistent with others who reported no effect of OXA during the 0- to 30-min postinjection time interval (Baldo and Kelley 2001). This lack of effect in the immediate postinjection period may be due to heightened physical activity due to handling during the injection process, as increased physical activity for up to 20 min postinjection in all treatment groups has been shown (Kotz et al. 2002).

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) (Moore and Card 1994; Harrington 1997). NPY neurons in the IGL project directly to the SCN (Harrington et al. 1987). Patterns of cellular activation of IGL NPY neurons are correlated with activity patterns in rodents (Janik et al. 1995; Smale et al. 2001; Webb et al. 2008). Manipulations which mimic release of NPY into the SCN result in changes in patterns of physical activity in rodents, and perturbations of the IGL or of NPY cells therein block these changes in activity (Johnson et al. 1989; Rusak et al. 1989; Biello et al. 1994; Huhman and Albers 1994; Wickland and Turek 1994). 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 (Nixon and Smale 2007; Cutler et al. 1999; Date et al. 1999; Peyron et al. 1998; Mintz et al. 2001; McGranaghan and Piggins 2001). Data suggest 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 (Nixon and Smale 2004, 2005). Patterns of cFos activation in orexin neurons are correlated with cFos expression patterns in NPY neurons of the IGL (Webb et al. 2008; Nixon and Smale 2005), 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 (Nixon and Smale 2005), 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 (Kiyashchenko et al. 2001) or the alpha gigantocellular reticular nucleus in the medioventral medullary region (Mileykovskiy et al. 2002) 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 (Peever et al. 2003). In contrast,

OXA microinjection into the pontine inhibitory area (Kiyashchenko et al. 2001) or the ventral gigantocellular reticular nucleus (Mileykovskiy et al. 2002) inhibits hindlimb muscle tone.

7 Orexin Effects on Sympathetic Outflow: Cardiovascular and Thermoregulatory Systems

Orexin effects on cardiovascular and thermoregulatory systems have been extensively reviewed (Shirasaka et al. 2002; Szekely et al. 2002, 2010; Ferguson and Samson 2003; Samson et al. 2005; Szekely 2006). Brain areas classically involved in thermoregulation and cardiovascular function receive orexin projections and express orexin receptors (Date et al. 1999, 2000; van den Pol 1999; van den Top et al. 2003), 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 (Shirasaka et al. 1999; Matsumura et al. 2001), plasma epinephrine (Shirasaka et al. 1999), noradrenaline release (Hirota et al. 2001), and firing rate of sympathetic nerves innervating the interscapular brown adipose tissue (iBAT) (Monda et al. 2004a, 2003, 2001), which would be expected to increase thermogenesis. Likewise, intrathecal OXA infusion (Antunes et al. 2001) and in vitro application (van den Top et al. 2003) stimulate sympathetic outflow. Tachycardia is observed following OXA in the lateral (Wang et al. 2001; Shirasaka et al. 1999; Monda et al. 2004a, 2003, 2001) and fourth ventricles (Zheng et al. 2005) or following infusion intracisternally (Chen et al. 2000) and intrathecally (Antunes et al. 2001), but not intravenously (Chen et al. 2000). Moreover, the pressor response to orexin is similar to the effect of orexin on heart rate (Samson et al. 1999; Shirasaka et al. 1999; Matsumura et al. 2001; Antunes et al. 2001; Chen et al. 2000). These sympathetic and cardiovascular effects are clearly mediated by multiple sites of action. Orexin A infusion into the rostral lateral ventral medulla (RVLM) (Chen et al. 2000), NTS (de Oliveira et al. 2003; Smith et al. 2002), Arc (Wang et al. 2003b), PVH (Monda et al. 2004a; Sato-Suzuki et al. 2002), and the diagonal band of Broca induces tachycardia (Monda et al. 2004a), and OXA in the RVLM (Chen et al. 2000) and NTS (de Oliveira et al. 2003; Smith et al. 2002) stimulate mean arterial pressure. In contrast, infusion into the nucleus ambiguous (de Oliveira and Ciriello 2003) or the subfornical organ induces bradycardia (Smith et al. 2007). While OXA in the nucleus ambiguous (de Oliveira and Ciriello 2003) has no effect on blood pressure, intrasubfornical organ OXA (Smith et al. 2007) 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 (Monda et al. 2001), iBAT (Monda et al. 2003, 2001), cutaneous (Monda et al. 2003), and core body temperature

(Zheng et al. 2005) following infusion into the lateral and fourth ventricles (Zheng et al. 2005), Arc (Wang et al. 2003b), and the diagonal band of Broca (Monda et al. 2004b). 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 (Verty et al. 2010). Furthermore, there is no effect of acute OXA on colonic temperature (Hagan et al. 1999) or of chronic OXA infusion on iBAT temperature (Haynes et al. 1999). 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 that orexin action is sympathoexcitatory, which would supplement energy expenditure due to orexin-mediated metabolic rate and physical activity.

8 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 postnatal 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 sensing areas and project mono- and multisynaptically 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 nonexercise energy expenditure. Orexin neurons project to and excite arcuate neuropeptide Y neurons (Horvath 2005). 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 (Nixon and Smale 2004; Wu et al. 2002). 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.

9 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 (Bray 2000), which is that of an inverse relationship between the two outputs of feeding and energy expenditure; for example, 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 (Bray 2000). 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, neuromodulators, neurotransmitters, and neurohormones) 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, and pharmacological studies and by studying obesity-prone and obesity-resistant models.

Narcolepsy in humans is accompanied by significantly reduced or absent orexin and significantly increased body mass (Thannickal et al. 2000; Nishino et al. 2001; Overeem et al. 2001), 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 (Hara et al. 2001). Orexin global overexpression 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 (Funato et al. 2009).

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 (Kotz et al. 2002, 2006; Teske et al. 2006). Obesity-resistant rat activity is also more sensitive to orexin (Kotz et al. 2002, 2006; Teske et al. 2006; Kiwaki et al. 2004; Novak et al. 2006a, 2010), 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 (Funato et al. 2009).

Thus, the energetic consequence of these two behavioral outputs, when added up on a caloric basis, results 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 are 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.

10 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 investigations (Brighton et al. 2004). 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 (Brighton et al. 2004; Mori et al. 2008; Miyazato et al. 2008; Mitchell et al. 2009); 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

(Minamino et al. 1985a, b). NMU is found at high levels within the intestine, specifically in neurons of the enteric nervous system (Brighton et al. 2004; Honzawa et al. 1990; Ballesta et al. 1988). 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 (Honzawa et al. 1987), 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 (Graham et al. 2003), and also dorsomedial hypothalamus (Ballesta et al. 1988; Graham et al. 2003; Nogueiras et al. 2006). Several nonhypothalamic regions also contain NMU-immunoreactive neurons and fibers, however, including hindbrain regions important in arousal and energy balance that are also responsive to CCK (Honzawa et al. 1987; Ivanov et al. 2004). 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 (Rucinski et al. 2007; Peier et al. 2009).

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 (Raddatz et al. 2000; Shan et al. 2000). 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 brain stem (Graham et al. 2003; Ivanov et al. 2004; Howard et al. 2000; Guan et al. 2001). Other brain systems also contain neuromedin binding or NMR2, including the hippocampus (Ivanov et al. 2004; Guan et al. 2001; Mangold et al. 2008; Zhang et al. 2010). NMR1 has also been identified in brain regions, including the amygdala (Gartlon et al. 2004). 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 (Gartlon et al. 2004; Ivanov et al. 2002; Niimi et al. 2001b). Though the hypothalamic regions and, to a lesser extent, the hindbrain regions, have received attention in functional behavioral or physiological studies (Ivanov et al. 2004; Novak et al. 2007, 2006b; Yokota et al. 2004; Wren et al. 2002; Thompson et al. 2004; Qiu et al. 2003, 2005), 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 (Semjonous et al. 2009; Howard et al. 2000; Ivanov et al. 2002; Wren et al. 2002). Activation of neuromedin receptors decreases food intake and

increases energy expenditure and physical activity (Semjonous et al. 2009; Peier et al. 2009; Novak et al. 2007, 2006b; Wren et al. 2002; Nakazato et al. 2000). Moreover, commonalities in the behavioral and energetic actions of neuromedins and homologous peptides can be seen in nonmammals, even invertebrates (Maruyama et al. 2011, 2008; Bader et al. 2007; Kamisoyama et al. 2007; Tachibana et al. 2010a, b; Yayou et al. 2009). It has been hypothesized that one action of leptin is to stimulate the release of NMU, through which it exerts its actions on metabolism (Graham et al. 2003; Nogueiras et al. 2006; Wren et al. 2002; Jethwa et al. 2005). Transgenic overexpression of NMU in mice leads to hypophagia and leanness (Kowalski et al. 2005), and deletion of the gene for NMU results in obesity, hyperphagia, and decreased physical activity (Hanada et al. 2004). Some have found that mice lacking the NMUR2 gene do not show this phenotype (Zeng et al. 2006), but others have found that NMUR2-deficient mice are lean, hypophagic, and somewhat resistant to weight and fat gain on a high-fat diet (Peier et al. 2009). In humans, variants in the gene encoding pro-NMU are associated with obesity (Hainerova et al. 2006). 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 (Thompson et al. 2004). In contrast, chronic central (i.c.v.) infusions of NMU using osmotic minipumps significantly suppressed energy intake, body weight, and adiposity, (Peier et al. 2009) though this effect may depend on the diet (Egecioglu et al. 2009).

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 (Novak et al. 2006b; Novak and Levine 2007; Sutton et al. 1982). In fact, neuromedins are likely to be one important component that activates brain CRH to affect behavior (Yokota et al. 2004; Wren et al. 2002; Hanada et al. 2001, 2003); CRH also appears to be necessary for some of the behavioral effects of NMU (Hanada et al. 2003). 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) (Sutton et al. 1982; Hanada et al. 2001; Jaszberenyi et al. 2007). In fact, brain neuromedins affect several other functions and behaviors besides food intake, including reproduction, the sleep/wake cycle (Ahnaou and Drinkenburg 2011), hippocampal and memory function (Zhang et al. 2010; Iwai et al. 2008), sympathetic outflow (Tanida et al. 2009), reproductive and stress axis function (Ivanov et al. 2002; Thompson et al. 2004; Hanada et al. 2004; Jaszberenyi et al. 2007; Jethwa et al. 2006; Fukue et al. 2006; Yang et al. 2010), prolactin secretion (Gartlon et al. 2004), pain sensitivity (Zeng et al. 2006), brain oxytocin and vasopressin systems (Niimi et al. 2001b; Qiu et al. 2005; Sakamoto et al. 2008, 2007), and bone remodeling (Sato et al. 2007).

11 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 orexins neurons is in a highly focused place in the lateral hypothalamus and perifornical areas. Work by Thorpe and Kotz (Kotz 2006) 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 the 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 it is 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 and as a contributor to the phenotypic output of other domains as well.

12 Orexin and Neuromedin Receptors as Therapeutic Targets

The pharmacologic efficacy of selective and dual orexin receptor 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 (Scammell and Winrow 2011). 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 and 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.

12.1 Orexin 1 Receptor Antagonists

The first commercially available selective orexin 1 receptor antagonist, SB-334867, has been shown to reverse orexin A-induced feeding (Haynes et al. 2000), physical activity-induced thermogenesis (Kiwaki et al. 2004), grooming (Duxon et al. 2001), sympathetic activity (Hirota et al. 2003), and arousal (Smith et al. 2003), as well as the delay in the normal transition from eating to resting (behavioral satiety sequence) induced by orexin A (Rodgers et al. 2001). Selective blockade of orexin 1 receptors by SB-334867 also attenuated orexin B-stimulated physical activity (Jones et al. 2001). In contrast, SB-408124 had no effect on sleep, physical activity, or body temperature after peripheral administration (Dugovic et al. 2009). Other selective orexin 1 receptor antagonists including SB-410220 (Langmead et al. 2004) and diaryl urea analogues of SB-334867 (Perrey et al. 2011) have been described pharmacologically; however, behavioral effects have yet to determined.

12.2 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-ylmethyl-acetamide (EMPA) reduced dark cycle basal physical activity, and [Ala^{11}, D-Leu^{15}], orexin B-stimulated physical activity (Malherbe et al. 2009). Peripheral infusion of JNJ-10397049 promoted sleep and reduced basal physical activity and body temperature in the light/dark cycle (Dugovic et al. 2009).

12.3 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 (Brisbare-Roch et al. 2007). Oral almorexant (ACT-078573) 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 (Li and Nattie 2010). Additional biocomparison, tolerability, pharmacokinetic, and pharmacodynamic tests in humans suggest that almorexant may be promising for treatment of insomnia (Hoch et al. 2011; Hoever et al. 2010).

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 (Winrow et al. 2011). Like its predecessor (Bergman et al. 2008), DORA-1 promoted sleep, reduced basal physical activity, and reduced physical activity stimulated by [Ala^{11}, D-Leu^{15}] orexin B and amphetamine (Winrow et al. 2010). In a similar manner, another dual orexin receptor antagonist based on a 1,4-diazepane central scaffold reduced basal dark cycle physical activity (Whitman et al. 2009). From these studies, DORA-5 was developed and was shown to reduce home cage physical activity and increase sleep after oral administration (Whitman et al. 2009). The clinical efficacy of another dual receptor antagonist, SB-674042, has also been demonstrated for insomnia (Reviewed in Scammell and Winrow 2011).

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 (Liu et al. 2009). 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.

13 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 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 neurons 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 brain stem. 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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