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Hypocretins/orexins as integrators of physiological information: lessons from mutant animals

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Summary The hypocretins/orexins (hcrts) are two recently described neuropeptides derived from the same precursor and expressed in a few thousand neurons in the perifornical area of the lateral hypothalamus, which project throughout the brain. The hypocretins bind to two G-protein coupled receptors with different selective affinities. Positional cloning of the gene responsible for a canine model of narcolepsy revealed that this disease is caused by mutations in hypocretin receptor type 2. Parallel studies with hypocretin/orexin knockout mice showed behavioral arrests reminiscent of narcolepsy-like attacks. Narcoleptic patients have decreased hypocretin-containing neurons suggesting that narcolepsy in humans is caused by selective neurodegeneration of hypocretinergic neurons. Additional functions for the hypocretins on regulation of energy balance neuroendocrine release and sympathetic outflow have been described. Here we review studies in humans and mutant animals that have provided clues about the functions of the hypocretinergic system, which appear to involve the coherent regulation of networks that dictate the states of arousal.

THE HYPOCRETINS (OREXINS): TWO HYPOTHALAMUS-SPECIFIC PEPTIDES

Analysis of the expression patterns of subtracted hypothalamus-enriched sequences (Gautvik et al., 1996) revealed one that was expressed exclusively by a bilaterally symmetric structure within the posterior hypothalamus. The respective cDNA sequence encoded a 130-residue putative secretory protein with an apparent signal sequence and 2 conserved sites for potential amidation. The two putative products of proteolysis had 13 amino acid identities across 19 residues (Figure 1). This region of one of the peptides contained a 7/7 match with secretin (residues marked with asterisks in figure 1), suggesting that the prepropeptide gave rise to two peptide products that were structurally related both to each other and to secretin. These putative peptides were named hypocretin (hcrt) 1 and 2 to reflect their hypothalamic origin and the similarity to the incretin neuropeptide family. Their precursor is, thus, preprohypocretin (de Lecea et al., 1998).

Sakurai and collaborators (Sakurai et al., 1998) prepared transfected cell lines stably expressing each of 50 orphan G protein-coupled receptors. Calcium fluxes were measured in response to fractions from tissue extracts. A peptide from rat brain extracts, called orexin A by those authors, corresponds to hypocretin-1 and was found to act at one of the receptors, and its sequence matched that of the C-terminally amidated form of hcrt 1 with the N-terminal glutamine derivatized as pyroglutamate. There were two intrachain disulfide bonds (Fig 1A). A less active peptide in the rat brain extract, orexin B was identical to hcrt2. This study demonstrated the presence and exact structures of the two mature hypocretin peptides in the brain. The non-amidated peptides (incorrectly referred to as hypocretins in Smart et al. (2000)) are not biologically active, and the disulfide bonds in hcrt1 may be important for some of its effects (Okumura et al., 2001), stabilizing the structure of the peptide. The hypocretins (hcrt 1 and hcrt 2) and orexins (OX-a and OX-b) are the products of the same gene and should be considered equivalent chemical entities.

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Hcrt1:          *EPLPDCCRQKTCSCRLYELLHGAGNHAAGILTL-NH2
Hcrt2:               RPGPPGLQGRLQRLLQANGNHAAGILTM-NH2
Secretin      HSDGTFTSKLSRLRDSARLQRLLQGLV HSDGTFTSK
VIP           HSDAVFTDNYTRLRKQMAVKKYLNSILN
PACAP27       HSDGIFTDSYSRYRKQMAVKKYLAAVLG
PACAP38       HSDGIFTDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNKG
Glucagon      HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
GLP-1         HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG
Hcrt1---------HAAGILT
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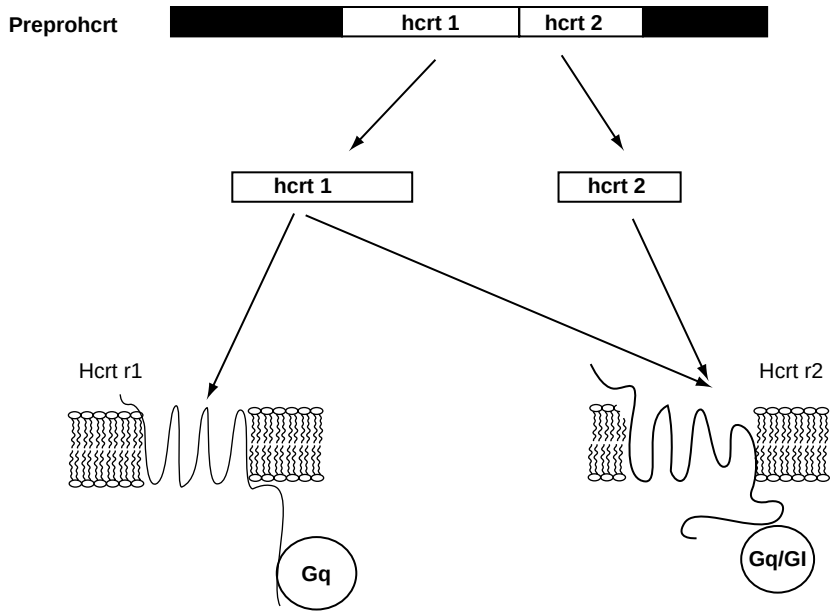

Fig. 1 Alignment of the two hypocretin peptides with members of the incretin peptide family. A. Amino acid alignment of incretin family members. Identical residues between hcrt1 and hcrt2 are indicated in bold. Identities between at least one of the hypocretins and at least one of the secretin family members (secretin, PACAP27, glucagon, GLP-1) are indicated by asterisks. The N-terminal regions of the secretin family members (shaded box) have been aligned with the C-terminal hypocretin residues to extend the region of identity. Disulfide bridges between cysteine residues and the pyroglutamate derivatization in hcrt1 were determined by Sakurai et al. (1998). B. Diagram of preprohypocretin, indicating the two peptides derived from the precursor and the relative affinities of the peptides to hcrt receptors 1 and 2.

The structure of hcrt 2 in solution has been determined by NMR (Lee et al., 1999), and consists of two alpha helices connected with a short linker. The hcrt 2 peptide shares a similar secondary-structural motif with human neuropeptide Y, and the amino acid residues that share similarity to the incretins, which constitute the large alpha helix of hcrt 2, span a large alpha helix in the secretin peptide (Alvarez and Sutcliffe, 2002), thus suggesting that the hypocretin peptides belong structurally to the incretin family of neuropeptides.

In addition to mammals, the sequence of the preprohypocretin mRNA has been determined in pufferfish (Alvarez and Sutcliffe, 2002), and *Xenopus* (Shibahara et al., 1999), and the distribution of hcrt immunoreactivity has been determined in the frog *Rana ridibunda* (Galas et al., 2001). The function of the hcrt peptides in lower vertebrates is unknown, although, interestingly, infusion of hcrt1 in goldfish increases food intake (Volkoff et al., 1999).

Hypocretin-containing cells project throughout the brain

Anatomical analysis of neuropeptide distribution is an important step towards understanding physiological function. Indeed, examination of hcrt mRNA distribution (see Figure 2) and the pattern of hcrt-like immunoreactivity has given key information about possible roles of these peptides in the brain.

Approximately 3,000 cell bodies immunoreactive for the hypocretins have been observed in the rat brain between the fornix and the mammillothalamic tracts. In addition to the hypothalamic neurons, a prominent network of axons can be located within the perifornical and posterior hypothalamus and beyond. Prominent fiber projections have been observed in apparent terminal fields within septal nuclei in the basal forebrain, the preoptic area, the paraventricular nucleus of the thalamus, the central gray, and the locus coeruleus. A complete mapping of these extensive projections from a relatively small number of neurons in the rat is given by Peyron and colleagues (Peyron et al., 1998). Similar immunoreactivity of cell bodies and terminals has been found in primates (Moore et al., 1998; Horvath et al., 1999). Electron microscopy revealed that hcrt immunoreactivity is associated with vesicles observed in the Golgi network, myelinated axons and at presynaptic terminals opposed to dendritic shafts.

The dense projections to the ventrolateral preoptic area, tuberomammillary nucleus, pontine reticular formation, laterodorsal tegmental area and locus coeruleus suggest involvement in states of arousal. Very strong hypocretin-immunoreactive projections have been described in regions of the spinal cord related to modulation of pain (van den Pol, 1999). In accordance with the wide distribution of hcrt terminals, intracerebroventricular administration of the hypocretins has been shown to affect sleep/wakefulness (Hagan et al., 1999; Bourgin et al., 2000; Piper et al., 2000), feeding (Sakurai, 1999), drinking (Kunii et al., 1999), sympathetic outflow (Samson et al., 1999; Shirasaka et al., 1999; Chen et al., 2000), neuroendocrine release (Pu et al., 1998; Mitsuma et al., 1999; Ida et al., 2000; Jaszberenyi et al., 2000; Russell et al., 2000; Mazzocchi et al., 2001; Russell et al., 2001), and learning (Telegdy and Adamik, 2002).

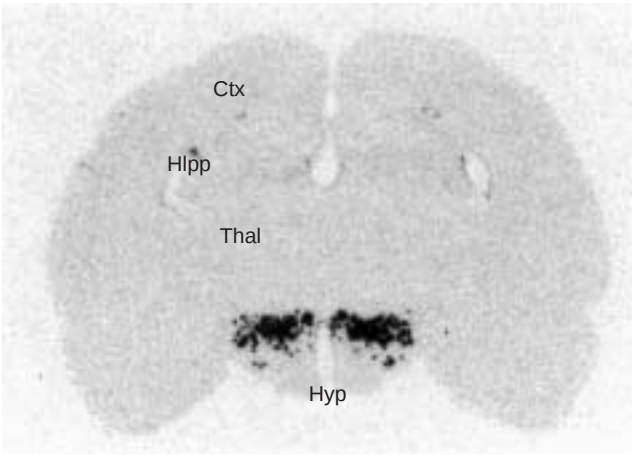

Fig. 2 The hypocretins are expressed in a few thousand cells in the rat brain. Detection of preprohcrt mRNA in large neurons in the dorsal-lateral hypothalamus by in situ hybridization to a coronal section from rat brain. (Ctx: cerebral cortex; Hipp: hippocampus; Thal: thalamus; Hyp: hypothalamus).

Hypocretins are neuroexcitatory

The putative structures of the hypocretins, their expression within the dorso-lateral hypothalamus and accumulation within vesicles at axon terminals suggested that they might have intercellular signaling activity. Indeed, bath application of nanomolar concentrations of synthetic hcrt2 to mature hypothalamic neurons evokes a substantial, but reversible, increase in the frequency of post synaptic currents (van den Pol et al., 1998). Similar results have been obtained when hcrt1 was applied on locus coeruleus slices (Hagan et al., 1999; Horvath et al., 1999). Hcrt2 has a potent effect at both presynaptic and postsynaptic receptors. Most synaptic activity in hypothalamic circuits is attributable to axonal release of GABA or glutamate. Hypocretin 1 and 2, acting directly at axon terminals, can increase the release of each of these amino acid transmitters, as seen with whole-cell patch-clamp recording (van den Pol et al., 1998). As in the adult central nervous system, the hcrts exert a profound excitatory influence on neuronal activity early in development. The peptides are present as early as 17-day rat embryos and they potently excite postsynaptic neurons at that age (Van Den Pol et al., 2001).

The hypocretin/orexin receptors

The initial orphan receptor, hcrtr 1, bound hcrt1 with high affinity, but hcrt2 with 100 to 1000-fold lower affinity. However, a related receptor, hcrtr2, identified by searching database entries with the hcrtr1 sequence, had high affinity for both hcrt2 and hcrt1 (Figure 1B) (Sakurai et al., 1998). Van den Pol and colleagues (van den Pol et al., 1998) have studied the second messenger systems involved in hypocretin signaling. Both hcrt1 and hcrt2 evoke rises in Ca^{2+}, as measured by fura-2 imaging, in about one third of hypothalamic neurons, probably by opening a plasma membrane calcium channel. Hypocretin responses are completely blocked by the PKC-specific inhibitor bisindolylmaleide, suggesting that hypocretin may work via G_q-activated PKC, resulting in phosphorylation of Ca^{2+} channels that has been reported to increase

Ca^{2+} conductance (Lund et al., 2000). More recent studies have shown that the hypocretins may be linked to the adenylyl cyclase pathway (Mazzocchi et al., 2001), likely via interaction of hcrtr2 with Gi (Karteris et al., 2001; Randeva et al., 2001). A non-peptide hcrtr1-selective receptor antagonist (1-(2-methylbenzoxazol-6-yl)-3-[1,5] naphthyridin-4-yl urea hydrochloride (SB-334867-A) (Smart et al., 2001), has been shown to inhibit hcrt-induced depolarization in rats (Rodgers et al., 2001; Soffin et al., 2002).

The mRNAs encoding the two receptors are both enriched in the brain and moderately abundant in the hypothalamus but display remarkably different distributions (Trivedi et al., 1998; Marcus et al., 2001). Hcrtr1 mRNA is expressed in many brain regions including the prefrontal and infralimbic cortex, hippocampus, paraventricular thalamic nucleus, ventromedial hypothalamic nucleus, dorsal raphe nucleus, and locus coeruleus. Immunocytochemical staining has also localized hcrtr1 protein to the same areas (Hervieu et al., 2001). Hcrtr2 mRNA is prominently expressed in a complementary distribution including the cerebral cortex, septal nuclei, hippocampus, medial thalamic groups, raphe nuclei, and many hypothalamic nuclei including the tuberomammillary nucleus, dorsomedial nucleus, paraventricular nucleus, and ventral premammillary nucleus. Immunocytochemical studies with antibodies raised against peptides derived from the receptors have confirmed this distribution (Cluderay et al., 2002). The differential expression pattern of hcrt receptors is consistent with the proposed multifaceted roles of hcrt in regulating homeostasis and may explain the unique role of the hcrtr2 receptor in regulating sleep state stability. The combined pattern of expression of these receptors is consistent with the map of hcrt-containing fibers, and suggests that the hypocretins modulate multiple neuronal circuits (see Figure 3).

Lessons from mutant dogs and mice

A set of recent papers have placed the hcrtergic neurons as the system responsible for narcolepsy and the regulation of REM sleep. Narcolepsy is a sleep disorder that strikes 1:2000 adults, appears between the age of 15 to 30, and shows four characteristic symptoms: i) excessive daytime sleepiness with irresistible sleep attacks during the day; ii) cataplexy (brief episodes of muscle weakness or paralysis precipitated by strong emotions such as laughter or surprise); iii) sleep paralysis, a symptom considered to be an abnormal episode of REM sleep atonia, in which the patient suddenly finds himself unable to move for a few minutes, most often upon falling asleep or waking up, and; iv) hypnagogic hallucinations, or dream-like images that occur at sleep onset. It has been known for some time that the disabling symptoms of narcolepsy are pathological equivalents of REM sleep (Mitler et al., 1990).

For more than a decade, Emmanuel Mignot's group has been characterizing the genetics of a canine form of narcolepsy that occurs in a colony of Doberman Pinschers, and is inherited as an autosomal recessive, fully penetrant

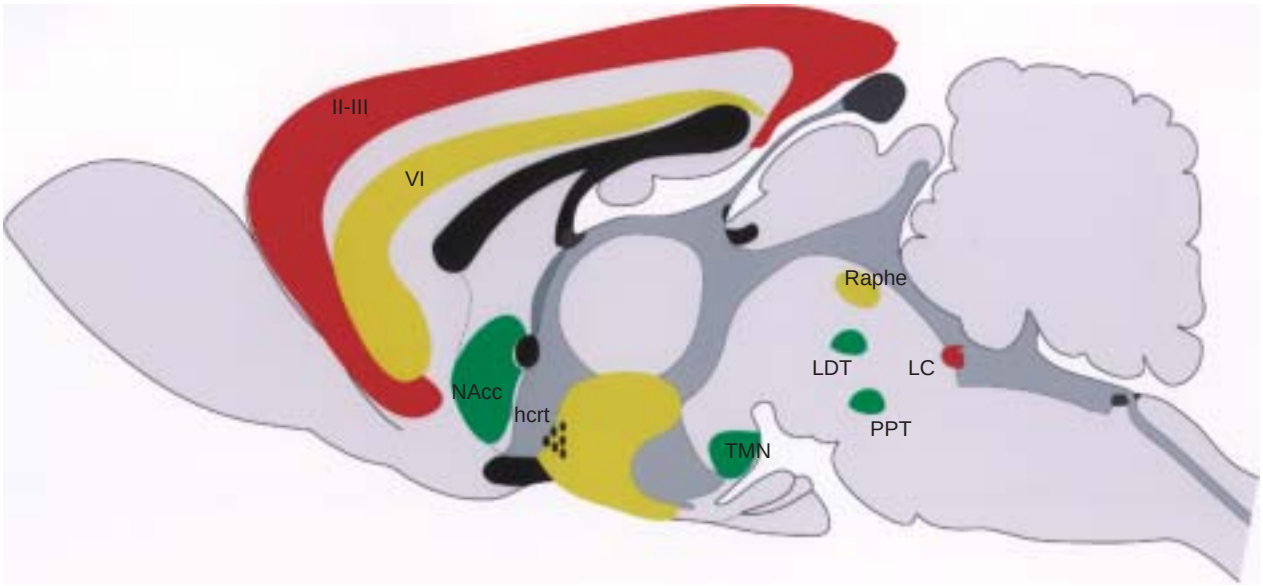

Fig. 3 Schematic of the distribution of hcrt receptors. Hcrt 1 expression is shown in red, hcrtr2 in green and the areas in which hcrtr1 and hcrtr2 overlap are shown in yellow. The cell bodies that express hcrt are indicated in the ventral hypothalamus. (abbreviations NAcc: nucleus accumbens; TMN: tuberomammilary nucleus; LDT: laterodorsal tegmentum; PPT: pedunculo pontine tegmentum; LC: locus coeruleus).

gene. Fine mapping and cloning of the canine narcolepsy (*canarc*) gene was accomplished with several narcoleptic individuals from Doberman families, and with the help of human bacterial artificial chromosomes from a region that shares synteny with canine chromosome 12 (Lin et al., 1999). In narcoleptic Dobermans, the *canarc* gene was found to be the gene encoding hcrtr2, and the structure of the mutated gene revealed an insertion of a a short interspersed repeat (SINE element) in the third intron that caused aberrant splicing of canine hcrtr2 mRNA and resulted in a truncated protein (Lin et al., 1999). Analysis of the gene in a colony of narcoleptic Labradors revealed that hcrtr 2 contained another mutation which also resulted in the premature termination of the protein. Administration of hcrt-1 in hcrtr2-deficient dogs does not have any effect on sleep architecture, suggesting a primary effect of hcrt2 in the maintenance of the awake state (Fujiki et al., 2001) although other reports have shown that systemic administration of hcrt 1 to hcrtr 2 deficient dogs improves the symptoms associated with narcolepsy (John et al., 2000). Partial destruction of hcrtr2-containing neurons after administration of hcrt2-saporin into the lateral hypothalamus of rats produces a narcolepsy-like behavior in adult animals (Gerashchenko et al., 2001), suggesting that hcrt neurons contain express hcrtr2 autoreceptors, and further supporting that hcrtr2 is the key receptor for the onset of narcolepsy.

Sleep recordings of mice lacking preprohcrt have shown increased amounts of slow wave sleep during the dark period, as well as wake/REM transitions, a landmark of narcolepsy. Moreover, hcrt knock-out mice exhibit periods of behavioral arrest during the dark phase, which may be related to narcoleptic attacks in humans. Interestingly, mice lacking hypocretin receptor 2 also show narcolepsy-like attacks, although they are less severe than those observed in the ligand knockout (Tokita et al., 2001). In contrast, sleep/wakefulness is normal in mice deficient in hert receptor 1 (Kisanuki et al., 2000). However, hcrtr1 and hcrtr2 double mutant mice display a phenotype which is indistinguishable from the hcrt knock out mouse, strongly suggesting that the hcrt peptides do not bind to other receptors than hcrtr1 and hcrtr2.

Transgenic expression of ataxin-3 in hcrt cells in mice produces a complete loss of these neurons at 15 weeks of age (Hara et al., 2001). The N-terminal truncated cDNA for human ataxin-3 used as the transgene in this study, was isolated from a patient with Machado-Joseph disease, includes abnormally expended polyglutamine repeats, and has been shown to induce apoptosis in many cell types when expressed exogenously. Hcrt-ataxin3 transgenic mice exhibit sleep abnormalities and behavioral arrests during the dark period as early as 6 weeks of age. This model is closer to the human disease, since the onset of narcolepsy occurs most frequently during adolescence. Only slight differences discriminate the sleep/wakefulness cycle of mice lacking pre-prohcrt and mice with ablation of hcrt neurons, suggesting that the hcrt peptides are critical to induce narcoleptic symptoms. Interestingly, hcrt-ataxin transgenic mice are hypophagic, hypoactive and become obese, indicating an imbalance in energy storage/consumption. Such a phenotype, which is less severe in hcrt knock-out mice, suggests that the deficiency in neurotransmitters normally co-expressed with hcrt such as dynorphin (Chou et al., 2001), may play a key role in energy homeostasis and food intake. Interestingly, there is a significant link between narcolepsy and obesity in humans (Schuld et al., 2000; Dahmen et al., 2001).

Another interesting lesson from mutant animals comes from studies assessing the effectiveness of the treatment of narcolepsy with amphetamines and other stimulants such as modafinil. Infusion of the stimulants in both controls and hcrtr2-deficient narcoleptic dogs results in increased wakefulness. Further, mutant mice deficient in the dopamine transporter show alterations in their sleep architecture and are insensitive to modafinil or amphetamine thus indicating that these compounds act by increasing dopamine levels, independently of hcrt activation (Wisor et al., 2001)

The hypocretins in human narcolepsy

The finding that canine narcolepsy is caused by mutations in hcrtr2 led to the examination of the hcrt system in human narcolepsy. While polymorphism studies revealed a single mutation in a rare form of early onset narcolepsy (Peyron et al., 2000), it became clear that mutations in hcrt receptors were not the cause of the human disease. However, measurements of the concentrations of hcrt1 peptide in the CSF of narcoleptic patients revealed that affected individuals had extremely low levels of hcrt1 compared to matched controls (Nishino et al., 2000). Further studies have shown that hcrt mRNA and peptide immunoreactivities are greatly diminished, if not absent, in narcoleptic patients (Peyron et al., 2000; Thannickal et al., 2000). Interestingly, some samples from narcoleptic patients displayed severe astrocytosis in the hypothalamus, although MCH-containing neurons, which are in close proximity to hcrt neurons were spared (Thannickal et al., 2000). These results, together with the previously established HLA association in narcolepsy (Langdon et al., 1984) suggest that the human disease is caused by an autoimmune attack that causes the specific destruction of hcrt neurons (Mignot et al., 2001).

The hypocretins in normal sleep/wake cycle

Despite major advances linking hcrt to narcolepsy, the precise role of hcrts in normal sleep is still a matter of

debate. Intracerebroventricular administration of hcrt1 produces an increase in wakefulness at the expense of non-REM sleep and a remarkable decrease in REM sleep (Hagan et al., 1999).

The actions of hcrt on sleep may be integrated into the reciprocal interaction model of REM sleep generation by McCarley and Hobson (1975). This model considers two populations of neurons: REM-off cells, which are silent during REM sleep, and REM-on neurons, which generate REM sleep bouts. REM-off cells, which include noradrenergic neurons of the LC, serotoninergic neurons of the raphe nucleus and the histaminergic neurons of the tuberomammilary nucleus (TMN), are highly active during wake and silent during REM sleep. During wakefulness, REM-off neurons inhibit REM-on cells, which include cholinergic neurons of the laterodorsal tegmentum and pedunculo pontine nucleus (LDT/PPT). During REM sleep, REM-on cells show a higher activity, after the inhibitory action of REM-off cells is removed. Considering the wake-promoting properties of hcrt1, it has been suggested that hcrt increases arousal and inhibits REM sleep by activating REM-off cells, in particular the noradrenergic ones in the LC which receive the densest hcrt innervation (Peyron et al., 1998; Date et al., 1999). This hypothesis is in line with *in vitro* and *in vivo* experiments which have shown that hcrt1 excites this cell population (Hagan et al., 1999; Horvath et al., 1999; Bourgin et al., 2000). Further, local administration of hcrt1 promotes wakefulness and suppresses REM sleep (Bourgin et al., 2000). Several studies have revealed the ability of hcrt to excite other REM-off neurons (Bayer et al., 2001; Brown et al., 2001; Eriksson et al., 2001; Huang et al., 2001), as well as REM-on cells in the LDT/PPT (Xi et al., 2001) and cholinergic neurons in the basal forebrain (Espana et al., 2001; Thakkar et al., 2001). Part of the wake promoting effects of hcrt seem to be mediated by histaminergic neurons in the tuberomammilary nucleus, as histamine H1 receptor knockout mice are impervious to hcrt administration (Huang et al., 2001). Clearly, similar analyses in mutant animals with alterations in specific neurotransmitter systems will lead to a better understanding of the interaction of the hcrts with the sleep/wake circuitry.

Two alternative models have been proposed to integrate the activity of hcrt neurons in the reciprocal inhibitory model for REM sleep regulation. Mignot and collaborators (Hungs and Mignot, 2001) considered hcrt neurons as wake neurons. During arousal, hcrt neurons are highly active stimulating both REM-off and REM-on neurons leading to the awake state. In contrast, during REM sleep, hcrt neurons exhibit minimal activity, reducing the firing

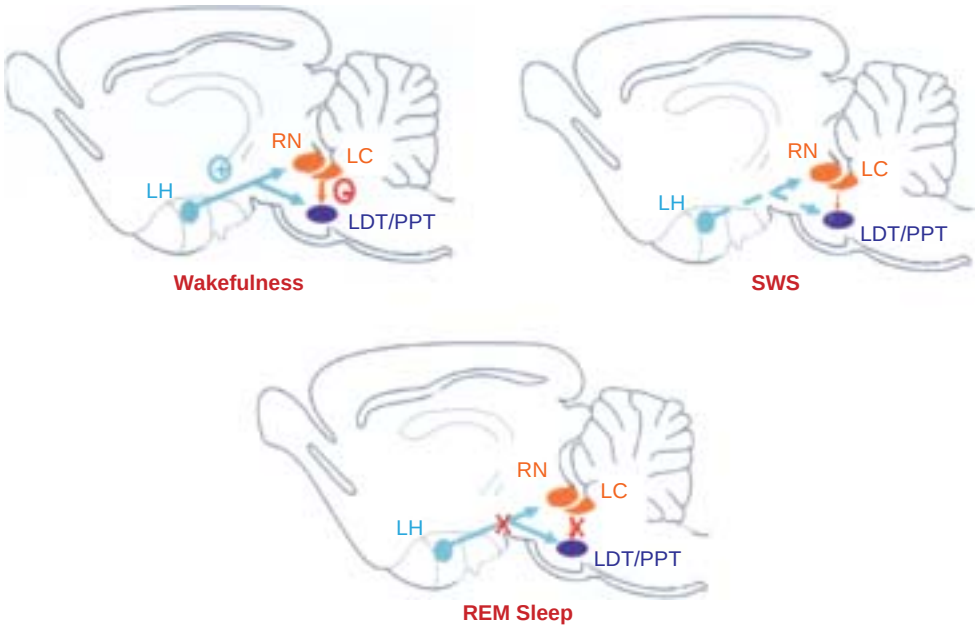

Fig. 4 Model of the states of vigilance defined by the activity of neurons in the lateral hypothalamus (modified from Hungs and Mignot, 2001). During wakefulness, hcrt neurons in the lateral hypothalamus (LH) excite both REM-on and REM-off cells, and REM-off cells strongly inhibit REM-on neurons. During slow wave sleep, the activity of REM-off cells is sufficient to inhibit REM-on neurons, despite lower firing activity of hcrt neurons. During REM sleep, hcrt neurons are silent, allowing REM-on neurons to initiate a REM sleep bout. It is important to note that hcrt neurons are mainly involved in the consolidation of states of vigilance and are important in the transition between sleep states, but are not required to promote wakefulness.

of REM-off neurons and subsequently activating REM-on neurons (Figure 4). In an alternative model Kilduff and Peyron (2000) proposed that hcrt cells are wake-on/REM-on neurons. According to this model, hcrt neurons drive the tonus of both REM-on and REM-off neurons during wakefulness. However, during REM sleep, REM-off cells will be inhibited by GABAergic neurons from the periaqueductal gray and disinhibit REM-on cells.

Investigating the activity of hcrt neurons during states of vigilance is therefore essential to understand the physiological role of hcrt in the regulation of sleep/wakefulness. Several studies correlate hcrt release with the sleep/wakefulness cycle. In freely moving rats, extracellular hcrt levels have been shown to be elevated in the lateral hypothalamus and the medial hypothalamus during the dark phase (active period) (Yoshida et al., 2001). Prolonged waking produced by pharmacological and instrumental sleep deprivations produces an increase in extracellular hcrt levels or c-fos/hcrt mRNA positive cells (Scammell et al., 2000; Estabrooke et al., 2001; Yoshida et al., 2001). Furthermore, Yoshida et al. (2001) found no correlation between hcrt levels and wake or sleep amounts, suggesting that hcrt may be primarily related to the regulation of the transitions between states of vigilance, rather than a particular sleep/wake stage. Some electrophysiological studies investigated the activity of neurons in the lateral hypothalamus in parallel with sleep recording in awake rats. Two cell types have been identified in the lateral hypothalamus: wake-on/REM-on neurons and REM-off cells indicating that LH neurons, which include hcrt neurons, exhibit a discharge pattern that correlates with arousal and sleep (Alam et al., 2002). However, it is difficult to correlate hcrtergic activity during the states of vigilance without a neurochemical characterization of these cell populations.

Are the hypocretins orexigenic?

Stereotactic ablation and physiological studies have previously implicated the dorsal-lateral hypothalamus in several homoeostatic processes, including feeding behavior, blood pressure, thermoregulation and arousal (Hetherington and Ranson, 1940; Anand and Brobeck, 1951; Reynolds, 1965; Donovan, 1966). Sakurai and colleagues (1998) demonstrated that intracerebroventricular administration of either peptide increased food consumption in rats. Furthermore, rats fasted for 48 hours increased the concentration of hcrt mRNA by 2.4 fold (Sakurai et al., 1998).

Hcrt-immunoreactive fibers make synaptic contacts with neurons in the arcuate nucleus that contain NPY, an important orexigenic peptide, and with POMC neurons, which produce α-MSH, raising the hypothesis that hcrts modulate feeding indirectly (Broberger et al., 1998; Elias et al., 1998). Indeed, pretreatment of rats with a corticotrophin releasing factor (CRF) antagonist increases the food enhancing effect of hcrt, and this can be blocked by NPY antagonists, suggesting that the hcrt-increase in food intake is mediated by NPY (Ida et al., 2000).

Hcrt-containing neurons express leptin receptors and also contain immunoreactivity for STAT 3, a transcription factor which is known to mediate leptin actions (Hakansson et al., 1999; Funahashi et al., 2000). Fasting-induced preprohcrt mRNA concentration increases are blocked by injection of leptin (Lopez et al., 2000) and preprohcrt mRNA expression is down regulated in leptin deficient *ob/ob* mice (Yamamoto et al., 2000). These data suggest that hcrt neurons receive input form the metabolic state by sensing the concentration of leptin. Hcrt neurons receive inputs from NPY- and AGRP-positive neurons in the arcuate nucleus (Horvath et al., 1998). Also supporting the idea that the hcrts are involved in feeding is the co-localization of hcrt immunoreactivity and the expression of *c-fos* induced by fasting in the lateral hypothalamus (Swart et al., 2001). Central administration of hcrt has been shown to increase metabolic rate (Lubkin and Stricker-Krongrad, 1998). Moreover, hcrt neurons are sensitive to glucose (Nowak et al., 2000; Cai et al., 2001; Muroya et al., 2001) and activated by glucopenia. The most solid evidence supporting a role of hcrt neurons on energy balance comes from the hcrt-ataxin3 mice, which are hypophagic and obese (Hara et al., 2001). This result demonstrates that hcrt neurons are part of the circuit that modulates energy storage, although the role of the hcrt peptides in energy homoestasis is less clear. In this regard, conflicting reports have shown increase and decrease body temperature upon injection of hcrt1 and hcrt2 (Balasko et al., 1999; Yoshimichi et al., 2001; Sze'kely et al., 2002), and very modest effect of food intake in hcrt knock out mice (Willie et al., 2001).

Together, these results suggest a complex circuitry of appetite controlling signaling molecules in the arcuate and lateral hypothalamus, in which hcrt neurons may play an integrating role.

The hypocretins affect multiple systems

In addition to their demonstrated role on sleep regulation, the hcrts have been associated with increases in sympathetic outflow and the regulation of neuroendocrine release. Intracerebroventricular administration of hcrt1 and 2 produce a significant increase in mean arterial blood pressure and heart rate (Samson et al., 1999; Shirasaka et al., 1999). These effects are also observed upon intracisternal injection of the peptides (Chen et al., 2000; Machado et al., 2002), suggesting that the hcrts affect sympathetic outflow by their projections to the spinal

cord (van den Pol, 1999). Also related to the spinal cord projections may be the analgesic properties observed upon injection of hcrt peptides (Bingham et al., 2001). The effects of the hcrts on cardiovascular parameters are consistent with their wake-promoting effects.

The effects of hcrts on neuroendocrine function have been well documented (Jaszberenyi et al., 2000; Kuru et al., 2000; Jaszberenyi et al., 2001; Samson et al., 2002). Hcrt1 and 2 fibers, peptides and receptors have been described in the median eminence and pituitary (Date et al., 2000; Blanco et al., 2001). The thyroid axis is inhibited by the hcrt system (Mitsuma et al., 1999) and is possibly responsible for the effects of the peptides on thermoregulation. Central infusion of the hcrt peptides affects the hypothalamo-pituitary-adrenal axis, as measured by ACTH and plasma corticosterone levels. Infusion of hcrt inhibits the corticotroph-induced release of ACTH from pituitary (Samson and Taylor, 2001) and hcrtr 2 appears to be enriched in the zona reticularis and glomerularis of the adrenal gland, suggesting a role in adrenal steroid synthesis (Johren et al., 2001). Interestingly, expression of the hcrtr2 in the adrenal glands is much higher in males than in females (Johren et al., 2001).

The hcrts inhibit prolactin and growth hormone release (Hagan et al., 1999; Russell et al., 2000). Like NPY, hcrt1 and hcrt2 increase LH release in ovarian steroid-treated ovarectomized rats, but not in unprimed rats (Pu et al., 1998). The levels of hcrt1 appear to change significantly across the estrous cycle (Russell et al., 2001) suggesting that the hcrt neurons also integrate networks affecting reproduction.

A role for the hcrt in gastrointestinal function has also been proposed, based on intense hcrt-like immunocytochemical staining in the intestinal epithelium (Kirchgessner and Liu, 1999). However, these findings need confirmation in animal models in which hcrt peptides or hcrt neurons are ablated.

Other complex behaviors, such as stereotypy, have been associated with the hcrtergic system. Intracerebroventricular administration of hcrt1 induces grooming and stereotypic behavior, which can be antagonized with D1 or D2 dopamine receptor antagonists (Nakamura et al., 2000).

FINAL REMARKS

The use of knock out animals has been determinant in understanding the neurobiology of the hcrtergic system. From their discovery in 1998, more than 350 publications have uncovered multiple effects associated with hcrtergic activity, most remarkably, their association with the transition between states of vigilance. It is not difficult to speculate that hcrtergic neurons play a key integrating role of the physiological state of the organism by sensing the concentration of leptin and the activity of other neuronal types associated with metabolic balance (i.e. NPY-, POMC- and MCH-positive neurons). Hcrt neurons receive monosynaptic afferents from the suprachiasmatic nucleus (Abrahamson et al., 2001), and thus sense information from the circadian clock. The input integrated in hcrt neurons results in the control of the stability major circuits involved in arousal, including the noradrenergic and histaminergic systems. Deficiency of the hcrt peptides or neurons in narcolepsy disrupts a coherent output that results in impaired transition between wakefulness and sleep and loss of the stability of states of arousal. As in narcolepsy, the discovery of phenotypes associated with loss or malfunction of discrete populations of neurons may open new avenues on neuropsychiatric research.

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REFERENCES

Abrahamson EE, Leak RK, Moore RY (2001) The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. Neuroreport 12: 435–440.

Alam MN, Gong H, Alam T, Jaganath R, McGinty D, Szymusiak R (2002) Sleep-waking discharge patterns of neurons recorded in the rat perifornical lateral hypothalamic area. J Physiol 538: 619–631.

Alvarez CA, Sutcliffe JG (2002) Hypocretin is an early member of the incretin gene family. Neurosci Lett 324: 169–172.

Anand BK, Brobeck JR (1951) Localization of a feeding center in the hypothalamus of the rat. Proc Soc Exp Biol Med 77: 323–324.

Balasko M, Szelenyi Z, Szekely M (1999) Central thermoregulatory effects of neuropeptide Y and orexin A in rats. Acta Physiol Hung 86: 219–222.

Bayer L, Eggermann E, Serafin M, Saint-Mleux B, Machard D, Jones B, Muhlethaler M (2001) Orexins (hypocretins) directly excite tuberomammillary neurons. Eur J Neurosci 14: 1571–1575.

Bingham S, Davey PT, Babbs AJ, Irving EA, Sammons MJ, Wyles M, Jeffrey P, Cutler L, Riba I, Johns A, Porter RA, Upton N, Hunter AJ, Parsons AA (2001) Orexin-A, an hypothalamic peptide with analgesic properties. Pain 92: 81–90.

Blanco M, Lopez M, Garcia-Caballero T, Gallego R, Vazquez-Boquete A, Morel G, Senaris R, Casanueva F, Dieguez C, Beiras A (2001) Cellular localization of orexin receptors in human pituitary. J Clin Endocrinol Metab 86: 1616–1619.

Bourgin P, Huitrón-Reséndiz S, Spier A, Fabre V, Morte B, Criado J, Sutcliffe J, Henriksen S, de Lecea L (2000) Hypocretin-1 modulates REM sleep through activation of locus coeruleus neurons. J Neurosci 20: 7760–7765.

Broberger C, de Lecea L, Sutcliffe JG, Hökfelt T (1998) Hypocretin/orexin- and melanin-concentrating hormone expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to neuropeptide Y innervation. J Comp Neurol 402: 460–474.

Brown RE, Sergeeva OA, Eriksson KS, Haas SL (2001) Orexin A excites serotonergic neurons in the dorsal raphe nucleus of the rat. Neuropharmacology 40: 457–459.

Cai XJ, Evans ML, Lister CA, Leslie RA, Arch JR, Wilson S, Williams G (2001) Hypoglycemia activates orexin neurons and selectively increases hypothalamic orexin-B levels: responses inhibited by feeding and possibly mediated by the nucleus of the solitary tract. Diabetes 50: 105–112.

Chen CT, Hwang LL, Chang JK, Dun NJ (2000) Pressor effects of orexins injected intracisternally and to rostral ventrolateral medulla of anesthetized rats. Am J Physiol Regul Integr Comp Physiol 278: R692–697.

Chou TC, Lee CE, Lu J, Elmquist JK, Hara J, Willie JT, Beuckmann CT, Chemelli RM, Sakurai T, Yanagisawa M, Saper CB, Scammell TE (2001) Orexin (hypocretin) neurons contain dynorphin. J Neurosci 21: RC168.

Cluderay JE, Harrison DC, Hervieu GJ (2002) Protein distribution of the orexin-2 receptor in the rat central nervous system. Regul Pept 104: 131–144.

Dahmen N, Bierbrauer J, Kasten M (2001) Increased prevalence of obesity in narcoleptic patients and relatives. Eur Arch Psychiatry Clin Neurosci 251: 85–89.

Date Y, Mondal MS, Matsukura S, Ueta Y, Yamashita H, Kaiya H, Kangawa K, Nakazato M (2000) Distribution of orexin/hypocretin in the rat median eminence and pituitary. Brain Res Mol Brain Res 76: 1–6.

Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, Nakazato M (1999) Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proc Natl Acad Sci USA 96: 748–753.

de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 95: 322–327.

Donovan BT (1966) Experimental lesions of the hypothalamus. A critical survey with particular reference to endocrine effects. Br Med Bull 22: 249–253.

Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffman GE, Ollmann MM, Barsh GS, Sakurai T, Yanagisawa M, Elmquist JM (1998) Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. J Comp Neurol 402: 442–459.

Eriksson KS, Sergeeva O, Brown RE, Haas HL (2001) Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J Neurosci 21: 9273–9279.

Espana RA, Baldo BA, Kelley AE, Berridge CW (2001) Wake-promoting and sleep-suppressing actions of hypocretin (orexin): basal forebrain sites of action. Neuroscience 106: 699–715.

Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M, Saper CB, Scammell TE (2001) Fos expression in orexin neurons varies with behavioral state. J Neurosci 21: 1656–1662.

Fujiki N, Yoshida Y, Ripley B, Mignot E, Nishino S (2001) Effect of systemic and central administration of hypocretin 1 in narcoleptic (hcrt r2 mutated) and control dogs. Sleep 24: A96.

Funahashi H, Hori T, Shimoda Y, Mizushima H, Ryushi T, Katoh S, Shioda S (2000) Morphological evidence for neural interactions between leptin and orexin in the hypothalamus. Regul Pept 92: 31–35.

Galas L, Vaudry H, Braun B, Van Den Pol AN, De Lecea L, Sutcliffe JG, Chartrel N (2001) Immunohistochemical localization and biochemical characterization of hypocretin/orexin-related peptides in the central nervous system of the frog Rana ridibunda. J Comp Neurol 429: 242–252.

Gautvik KM, de Lecea L, Gautvik VT, Danielson PE, Tranque P, Dopazo A, Bloom FE, Sutcliffe JG (1996) Overview of the most prevalent hypothalamus-specific mRNAs, as identified by directional tag PCR subtraction. Proc Natl Acad Sci USA 93: 8733–8738.

Gerashchenko D, Kohls MD, Greco M, Waleh NS, Salin-Pascual R, Kilduff TS, Lappi DA, Shiromani PJ (2001) Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behavior in the rat. J Neurosci 21: 7273–7283.

Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DN, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N (1999) Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci USA 96: 10911–10916.

Hakansson M, De Lecea L, Sutcliffe JG, Yanagisawa M, Meister B (1999) Leptin receptor- and STAT3-immunoreactivities in Hypocretin/Orexin neurones of the lateral hypothalamus1. J Neuroendocrinol 11: 653–663.

Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 30: 345–354.

Hervieu GJ, Cluderay JE, Harrison DC, Roberts JC, Leslie RA (2001) Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. Neuroscience 103: 777–797.

Hetherington AW, Ranson SW (1940) Hypothalamic lesions and adiposity in the rat. Anat Rec 78: 149–172.

Horvath TL, Diano S, Van den Pol AN (1998) Hypocretin (orexin)-containing neurons make synaptic contact with arcuate nucleus NPY- and POMC-producing cells that express leptin receptors in the rodent and primate: novel hypothalamic circuit involved in energy homeostasis. Soc Neurosci Abstr 24: 12.

Horvath TL, Peyron C, Diano S, Ivanov A, Aston-Jones G, Kilduff TS, van Den Pol AN (1999) Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. J Comp Neurol 415: 145–159.

Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, Urade Y, Hayaishi O (2001) Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci USA 98: 9965–9970.

Hungs M, Mignot E (2001) Hypocretin/orexin, sleep and narcolepsy. Bioessays 23: 397–408.

Ida T, Nakahara K, Kuroiwa T, Fukui K, Nakazato M, Murakami T, Murakami N (2000) Both corticotropin releasing factor and neuropeptide Y are involved in the effect of orexin (hypocretin) on the food intake in rats. Neurosci Lett 293: 119–122.

Jaszberenyi M, Bujdoso E, Telegdy G (2001) The role of neuropeptide y in orexin-induced hypothalamic-pituitary-adrenal activation. J Neuroendocrinol 13: 438–441.

Jaszberenyi M, Bujdoso E, Pataki I, Telegdy G (2000) Effects of orexins on the hypothalamic-pituitary-adrenal system. J Neuroendocrinol 12: 1174–1178.

John J, Wu MF, Siegel JM (2000) Systemic administration of hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. Sleep Res Online 3: 23–28.

Johren O, Neidert SJ, Kummer M, Dendorfer A, Dominiak P (2001) Prepro-Orexin and Orexin Receptor mRNAs Are Differentially Expressed in Peripheral Tissues of Male and Female Rats. Endocrinology 142: 3324–3331.

Karteris E, Randeva HS, Grammatopoulos DK, Jaffe RB, Hillhouse EW (2001) Expression and coupling characteristics of the CRH and orexin type 2 receptors in human fetal adrenals. J Clin Endocrinol Metab 86: 4512–4519.

Kilduff TS, Peyron C (2000) The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. Trends Neurosci 23: 359–365.

Kirchgessner AL, Liu M (1999) Orexin synthesis and response in the gut. Neuron 24: 941–951.

Kisanuki YY, Chemelli RM, Sinton CM, Williams SC, Richardson JA, Hammer RE, Yanagisawa M (2000) The role of orexin receptor Type 1 (OX1R) in the regulation of sleep. Sleep 23 (Suppl): A91.

Kunii K, Yamanaka A, Nambu T, Matsuzaki I, Goto K, Sakurai T (1999) Orexins/hypocretins regulate drinking behaviour. Brain Res 842: 256–261.

Kuru M, Ueta Y, Serino R, Nakazato M, Yamamoto Y, Shibuya I, Yamashita H (2000) Centrally administered orexin/hypocretin activates HPA axis in rats. Neuroreport 11: 1977–1980.

Langdon N, Welsh KI, van Dam M, Vaughan RW, Parkes D (1984) Genetic markers in narcolepsy. Lancet 2: 1178–1180.

Lee JH, Bang E, Chae KJ, Kim JY, Lee DW, Lee W (1999) Solution structure of a new hypothalamic neuropeptide, human hypocretin- 2/orexin-B. Eur J Biochem 266: 831–839.

Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 98: 365–376.

Lopez M, Seoane L, Garcia MC, Lago F, Casanueva FF, Senaris R, Dieguez C (2000) Leptin regulation of prepro-orexin and orexin receptor mRNA levels in the hypothalamus. Biochem Biophys Res Commun 269: 41–45.

Lubkin M, Stricker-Krongrad A (1998) Independent Feeding and Metabolic Actions of Orexins in Mice. Biochem Biophys Res Commun 253: 241–245.

Lund PE, Shariatmadari R, Uustare A, Detheux M, Parmentier M, Kukkonen JP, Akerman KE (2000) The Orexin OX1 Receptor Activates a Novel Ca2 + Influx Pathway Necessary for Coupling to Phospholipase C. J Biol Chem 275: 30806–30812.

Machado BH, Bonagamba LG, Dun SL, Kwok EH, Dun NJ (2002) Pressor response to microinjection of orexin/hypocretin into rostral ventrolateral medulla of awake rats. Regul Pept 104: 75–81.

Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, Elmquist JK (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 435: 6–25.

Mazzocchi G, Malendowicz LK, Gottardo L, Aragona F, Nussdorfer GG (2001) Orexin A stimulates cortisol secretion from human adrenocortical cells through activation of the adenylate cyclase-dependent signaling cascade. J Clin Endocrinol Metab 86: 778–782.

McCarley RW, Hobson JA (1975) Neuronal excitability modulation over the sleep cycle: a structural and mathematical model. Science 189: 58–60.

Mignot E, Lin L, Rogers W, Honda Y, Qiu X, Lin X, Okun M, Hohjoh H, Miki T, Hsu S, Leffell M, Grumet F, Fernandez-Vina M, Honda M, Risch N (2001) Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. Am J Hum Genet 68: 686–699.

Mitler MM, Hajdukovic R, Erman M, Koziol JA (1990) Narcolepsy. J Clin Neurophysiol 7: 93–118.

Mitsuma T, Hirooka Y, Mori Y, Kayama M, Adachi K, Rhue N, Ping J, Nogimori T (1999) Effects of orexin A on thyrotropin-releasing hormone and thyrotropin secretion in rats. Horm Metab Res 31: 606–609.

Moore RY, Speh JC, van den Pol AN (1998) Hypocretin localization in the human hypothalamus. Soc Neurosci Abstr 24: 1918.

Muroya S, Uramura K, Sakurai T, Takigawa M, Yada T (2001) Lowering glucose concentrations increases cytosolic Ca(2 +) in orexin neurons of the rat lateral hypothalamus. Neurosci Lett 309: 165–168.

Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, Sakurai T (2000) Orexin-induced hyperlocomotion and stereotype are mediated by the dopaminergic system. Brain Res 873: 181–187.

Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E (2000) Hypocretin (orexin) deficiency in human narcolepsy [letter]. Lancet 355: 39–40.

Nowak KW, Mackowiak P, Switonska MM, Fabis M, Malendowicz LK (2000) Acute orexin effects on insulin secretion in the rat: in vivo and in vitro studies. Life Sci 66: 449–454.

Okumura T, Takeuchi S, Motomura W, Yamada H, Egashira Si S, Asahi S, Kanatani A, Ihara M, Kohgo Y (2001) Requirement of intact disulfide bonds in orexin-A-induced stimulation of gastric acid secretion that is mediated by OX1 receptor activation. Biochem Biophys Res Commun 280: 976–981.

Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS (1998) Neurons Containing Hypocretin (Orexin) Project to Multiple Neuronal Systems. J Neurosci 18: 9996–10015.

Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 6: 991–997.

Piper DC, Upton N, Smith MI, Hunter AJ (2000) The novel brain neuropeptide, orexin-A, modulates the sleep-wake cycle of rats. Eur J Neurosci 12: 726–730.

Pu S, Jain MR, Kalra PS, Kalra SP (1998) Orexins, a novel family of hypothalamic neuropeptides, modulate pituitary luteinizing hormone secretion in an ovarian steroid-dependent manner. Regul Pept 78: 133–136.

Randeva HS, Karteris E, Grammatopoulos D, Hillhouse EW (2001) Expression of orexin-A and functional orexin type 2 receptors in the human adult adrenals: implications for adrenal function and energy homeostasis. J Clin Endocrinol Metab 86: 4808–4813.

Reynolds RW (1965) Hypothalamic lesions and disinhibition of feeding. Science 150: 1322.

Rodgers RJ, Halford JC, Nunes de Souza RL, Canto de Souza AL, Piper DC, Arch JR, Upton N, Porter RA, Johns A, Blundell JE (2001) SB-334867, a selective orexin-1 receptor antagonist, enhances behavioural satiety and blocks the hyperphagic effect of orexin-A in rats. Eur J Neurosci 13: 1444–1452.

Russell SH, Small CJ, Kennedy AR, Stanley SA, Seth A, Murphy KG, Taheri S, Ghatei MA, Bloom SR (2001) Orexin A interactions in the hypothalamo-pituitary gonadal axis. Endocrinology 142: 5294–5302.

Russell SH, Kim MS, Small CJ, Abbott CR, Morgan DG, Taheri S, Murphy KG, Todd JF, Ghatei MA, Bloom SR (2000) Central administration of orexin A suppresses basal and domperidone stimulated plasma prolactin. J Neuroendocrinol 12: 1213–1218.

Sakurai T (1999) Orexins and orexin receptors: implication in feeding behavior. Regul Pept 85: 25–30.

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR,

Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M (*1998*) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell *92*: 573–585.

Samson WK, Taylor MM (2001) Hypocretin/orexin suppresses corticotroph responsiveness in vitro. Am J Physiol Regul Integr Comp Physiol 281: R1140–1145.

Samson WK, Taylor MM, Follwell M, Ferguson AV (2002) Orexin actions in hypothalamic paraventricular nucleus: physiological consequences and cellular correlates. Regul Pept 104: *97*–103.

Samson WK, Gosnell B, Chang JK, Resch ZT, Murphy TC (*1999*) Cardiovascular regulatory actions of the hypocretins in brain. Brain Res 831: 248–253.

Scammell TE, Estabrooke IV, McCarthy MT, Chemelli RM, Yanagisawa M, Miller MS, Saper CB (2000) Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 20: 8620–8628.

Schuld A, Hebebrand J, Geller F, Pollmacher T (2000) Increased body-mass index in patients with narcolepsy. Lancet 355: 1274–1275.

Shibahara M, Sakurai T, Nambu T, Takenouchi T, Iwaasa H, Egashira SI, Ihara M, Goto K (*1999*) Structure, tissue distribution, and pharmacological characterization of Xenopus orexins. Peptides 20: 1169–1176.

Shirasaka T, Nakazato M, Matsukura S, Takasaki M, Kannan H (*1999*) Sympathetic and cardiovascular actions of orexins in conscious rats. Am J Physiol 277: R1780–1785.

Smart D, Jerman JC, Brough SJ, Neville WA, Jewitt F, Porter RA (2000) The hypocretins are weak agonists at recombinant human orexin-1 and orexin-2 receptors. Br J Pharmacol 129: 1289–1291.

Smart D, Sabido-David C, Brough SJ, Jewitt F, Johns A, Porter RA, Jerman JC (2001) SB-334867-A: the first selective orexin-1 receptor antagonist. Br J Pharmacol 132: 1179–1182.

Soffin EM, Evans ML, Gill CH, Harries MH, Benham CD, Davies CH (2002) SB-334867-A antagonises orexin mediated excitation in the locus coeruleus. Neuropharmacology 42: 127–133.

Swart I, Michael Overton J, Houpt TA (2001) The effect of food deprivation and experimental diabetes on orexin and NPY mRNA levels. Peptides 22: 2175–2179.

Sze'kely M, Pe'terva'ri E, Balasko M, Herna'di I, Uzsoki B (2002) Effects of orexins on energy balance and thermoregulation. Regul Pept 104: 47–53.

Telegdy G, Adamik A (2002) The action of orexin A on passive avoidance learning. Involvement of transmitters. Regul Pept 104: 105–110.

Thakkar MM, Ramesh V, Strecker RE, McCarley RW (2001) Microdialysis perfusion of orexin-A in the basal forebrain increases wakefulness in freely behaving rats. Arch Ital Biol 139: 313–328.

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. Neuron 27: 469–474.

Tokita S, Chemelli RM, Willie JT, Yanagisawa M (2001) Behavioral characterization of orexin 2 receptor knock out mice. Sleep 24 (suppl): A21.

Trivedi P, Yu H, MacNeil DJ, Van der Ploeg LH, Guan XM (*1998*) Distribution of orexin receptor mRNA in the rat brain. FEBS Lett 438: 71–75.

van den Pol AN (*1999*) Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. J Neurosci 19: 3171–3182.

van Den Pol AN, Patrylo PR, Ghosh PK, Gao XB (2001) Lateral hypothalamus: Early developmental expression and response to hypocretin (orexin). J Comp Neurol 433: 349–363.

van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB (*1998*) Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. J Neurosci 18: *7962*–*7971*.

Volkoff H, Bjorklund JM, Peter RE (*1999*) Stimulation of feeding behavior and food consumption in the goldfish, Carassius auratus, by orexin-A and orexin-B. Brain Res 846: 204–209.

Willie JT, Chemelli RM, Sinton CM, Yanagisawa M (2001) To eat or to sleep? orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci 24: 429–458.

Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM (2001) Dopaminergic role in stimulant-induced wakefulness. J Neurosci 21: 1787–1794.

Xi M, Morales FR, Chase MH (2001) Effects on sleep and wakefulness of the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. Brain Res 901: 259–264.

Yamamoto Y, Ueta Y, Serino R, Nomura M, Shibuya I, Yamashita H (2000) Effects of food restriction on the hypothalamic prepro-orexin gene expression in genetically obese mice. Brain Res Bull 51: 515–521.

Yoshida Y, Fujiki N, Nakajima T, Ripley B, Matsumura H, Yoneda H, Mignot E, Nishino S (2001) Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activities. Eur J Neurosci 14: 1075–1081.

Yoshimichi G, Yoshimatsu H, Masaki T, Sakata T (2001) Orexin-A regulates body temperature in coordination with arousal status. Exp Biol Med (Maywood) 226: 468–476.