Modulation by the hypocretinergic/orexinergic neurotransmission system in sleep-wakefulness cycle states

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MODULATION BY THE HYPOCRETINERGIC/OREXINERGIC NEUROTRANSMISSION SYSTEM IN SLEEP-WAKEFULNESS CYCLE STATES

Summary. Introduction. *The hypocretins/orexins are neuropeptides synthesized by a small neuronal cell group located in the posterior and lateral hypothalamus. These peptides have been considered modulators of the sleep-wakefulness cycle since their discovery in 1998; the hypocretinergic/orexinergic system is very active during wakefulness. In addition, the absence of either these peptides or their receptors is associated to narcolepsy-cataplexy, a disease in which the sleep-wakefulness cycle is completely disorganized.* Development. *Hypocretinergic/orexinergic neurons directly activate the cerebral cortex and neuronal cell groups of the reticular activating system containing noradrenaline, serotonin, dopamine, acetylcholine and histamine, through which they may also indirectly activate the cerebral cortex and enhance wakefulness; as well, these neurons inhibit REM sleep generation in the ventral pontine tegmentum. The decrease in the activity of hypocretinergic/orexinergic neurons during sleep inhibits the aminergic and cholinergic neurons of the reticular activating core, decreasing cortical activation and renewing REM sleep generation in the ventral pontine tegmentum.* Conclusions. *Hypocretins/orexins modulate wakefulness and EEG activation in part through their actions on reticular core neurons projecting to the cortex and suppress REM sleep generation through inhibition of ventral pontine tegmentum neurons within the ventral oral pontine tegmentum. The hypoactivity of this system supports the sleep cycle fragmentation and general disorganization appearing in narcolepsy, as well as momentary interruption of wakefulness by REM sleep episodes. [REV NEUROL 2007; 45: 482-90]*

Key words. *Hypocretin. Hypothalamus. Narcolepsy. Orexin. REM. Sleep. Wakefulness.*

INTRODUCTION

Hypocretins/orexins (Hcrt/Ox) are a group of hypothalamic neuropeptides involved in sleep-wakefulness cycle regulation, among other functions. Since the Hcrt/Ox were discovered, many data have been gathered about their actions enhancing wakefulness and cortical EEG activation. It has been also proposed that these neuropeptides may inhibit or defacilitate structures involved in REM sleep generation during wakefulness. Cessation of activity by Hcrt/Ox neurons during REM seems to provoke activation of REM-enhancing structures within the brainstem reticular formation. Hcrt/Ox regulation of sleep-wakefulness states underlies the pathogenic bases of narcolepsy and, therefore, has direct clinical implications. The alteration of the hypocretinergic/orexinergic system in that disease produces a general disorganization of the sleep-wakefulness cycle.

Hcrt/Ox neurons may exert their wake-enhancing actions either by acting directly on the cerebral cortex or by activating subcortical wake-enhancing centers projecting widely to the cortical mantle. The locus coeruleus (LC), dorsal raphe nucleus (RDo), ventral tegmental area (VTA), tuberomammillary nucleus (TMN), laterodorsal (LDT) and pedunculopontine (PPT) tegmental nuclei, and basal forebrain (BF) are involved in both wakefulness and cortical activation maintenance, and are characterized by their diffuse direct projections to the cortex through extrathalamic pathways. The enhanced wakefulness produced by Hcrt/Ox neurons could be due in part to their actions on LC, RDo, VTA, TMN, PPT, LDT and BF.

With the understanding that RDo, LC, TMN, and VTA are wake-enhancing structures while the ventral portion of the pontine reticular nucleus (vRPO) is a REM-generating center, in this revision we review and update the existing information on the fundamental neurobiology of Hcrt/Ox and the modulation this peptidergic system exerts on sleep-wakefulness cycle states through these structures.

The exact mechanisms by which Hcrt/Ox facilitate cortical activation and wakefulness are far from being known at the present time, although a quite plausible hypothesis would be that Hcrt/Ox act either directly in the cortex [1] or indirectly through the activation of cortically-projecting neurons located in the brainstem or forebrain [2]. Most of the Hcrt/Ox neurons increase their discharge rates during arousal and decrease their activity during sleep [3].

HYPOCRETINS/OREXINS

In 1998 De Lecea's and Sakurai's groups independently described the existence of two peptides synthesized by hypothalamic neurons [4,5]. De Lecea et al. observed that these peptides are expressed only in the posterolateral hypothalamus (HPL) and were very similar to the secretin-related peptides, so they named them hypocretin-1 (Hcrt1) and hypocretin 2 (Hcrt2) [4]. Sakurai et al. reported that central administration of the peptides increased feeding and called them orexin A (OxA) and orexin B (OxB) [5].

Although Hcrt/Ox neurons are scarce, they have a profuse projection system to numerous brain regions involved in sleep-wakefulness cycle regulation. Among the main structures inner-

Accepted: 17.07.07

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This work was supported by grants of the Ministerio de Educación y Ciencia (Refs. BFI2003-00809 and BFU2006-07430). The authors thanks Ms Carol Warren for the linguistic correction of the final version.

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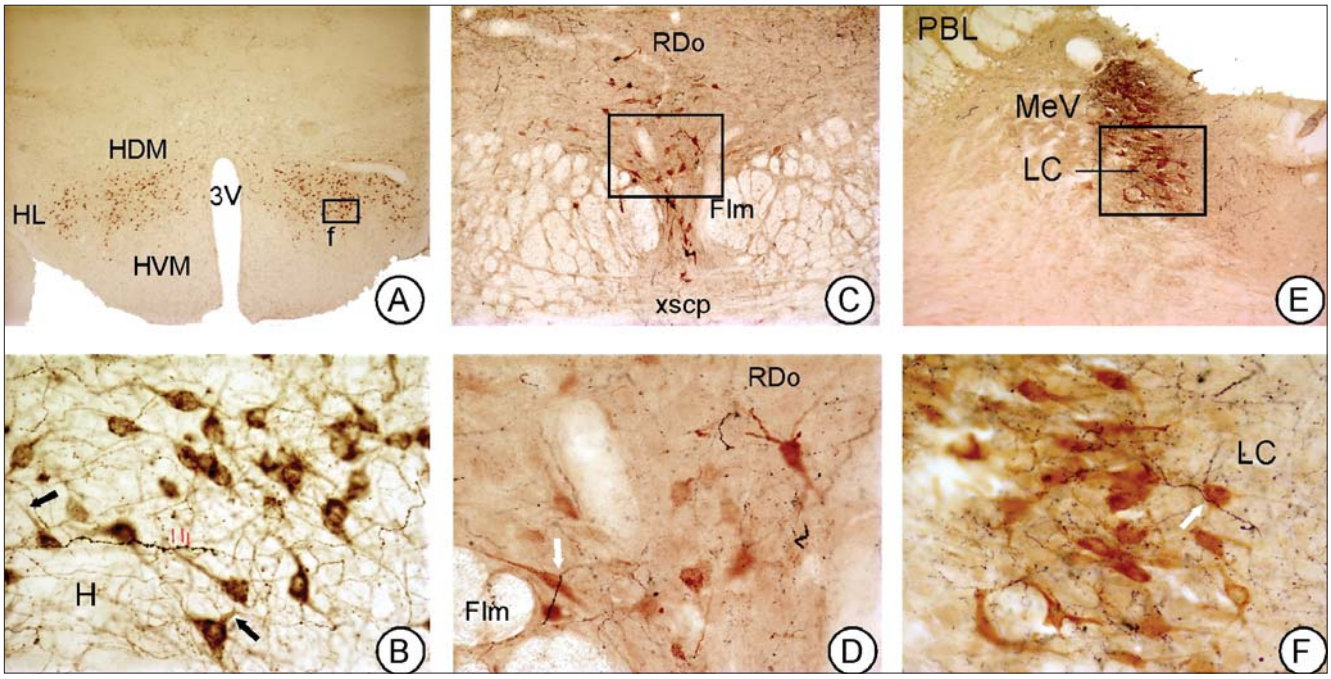

Figure 1. Orexin-A (OxA) immunoreactivity in coronal sections of the rat brain. A) Photomicrograph depicting OxA immunoreactive neurons in the posterolateral hypothalamus. B) Higher magnification of boxed area in A. The brown DAB reaction product fills the cytoplasm of some hypothalamic multipolar neurons. Red arrows indicate some varicosities in an OXA-immunolabeled fiber; black arrow points to a dendritic bifurcation within an OxA-imunoreactive neuron. C) Dual immunohistochemical staining of the dorsal raphe nucleus for OXA and fluorogold retrogradely traced from the medial frontal cortex. D) High magnification of the boxed area in C. The white arrow marks the superposition of an OXA-immunoreactive fiber (black reaction product) and an RDo neuron fillied with fluorogold (brown reaction product). E) Dual immunohistochemical staining of the locus coeruleus area for OXA and fluorogold retrogradely traced from the medial frontal cortex. F) High magnification of the boxed area in E. The white arrow indicates superposition of an OXA-immunoreactive varicose axon (black reaction product) and a fluorogold-immunolabeled neuron (brown reaction product), which therefore projects to the cortex. 3V: 3rd ventricle; f: fornix; Flm: medial longitudinal fasciculus; H: hypothalamus; HDM: dorsomedial hypothalamic nucleus; HL: lateral hypothalamus; HVM: ventromedial hypothalamic nucleus; LC: locus coeruleus; MeV: mesencephalic trigeminal nucleus; PBL: lateral parabrachial nucleus; RDo: dorsal raphe nucleus; xscp: decussation of the superior cerebellar peduncle.

vated by Hcrt/Ox neurons are the locus coeruleus, the dorsal raphe nucleus and the basal forebrain [6], all known to participate in arousal and cortical activation enhancement. Moreover, Hcrt/Ox neurons innervate the brainstem tegmentum, including the REM sleep inducing region located in the ventral portion of the oral pontine reticular nucleus (vRPO) [7].

The Hcrt/Ox neurons are symmetrically distributed in the dorsal and lateral hypothalamic areas [4,5], in the perifornical region localized between the fornix and the mammillothalamic tract (Fig. 1). After they were reported, it was discovered that Hcrt and Ox were the same peptides [8]. Both of them derived from the same precursor protein, called preprohypocretin (preproHcrt) [4] or preproorexin (preproOx) [5]. PreproHcrt/preproOx is formed by 131 amino acids in humans and rodents. It presents 3 proteolytic sites whose cleavage produces Hcrt1/OxA (33 amino acids) and Hcrt2/OxB (28 amino acids), each of which has glycine residues, an N-terminal pyroglutamyl residue and a C-terminal amidated residue [4,5]. Hcrt1/OxA differs from Hcrt2/OxB in that the former has two disulfide bonds, which confer a greater structural stability on it and allows Hcrt1/OxA to remain in cerebrospinal fluid (CSF) [9].

The Hcrt/Ox gene is located in chromosome 17q21-q24 [4,5]. Hcrt1/OxA and Hcrt2/OxB present an approximately 46% sequence homology depending on the species, with most differences occurring in the C-terminal. The Hcrt2/OxB sequence in humans and rodents seems to diverge in two amino acids, although some controversy still persists about this issue [4,5,10]. Hcrt1/OxA is identical in humans, rats, mice and sheep, and is not found in pineal gland, pituitary gland or most peripheral tissues [10,11].

There are about 15,000-80,000 Hcrt/Ox neurons in the rat brain; they have a diameter of 25-30 μm [4] and usually present an indented nucleus with a large nucleolus [6]. Hcrt/Ox-immunoreactive neurons show rather diverse morphology, mainly fusiform or multipolar [4,5]. At the subcellular level, Hcrt/Ox is localted in endoplasmic reticulum cisterns, Golgi complex and large-size dense core granular cytoplasmic vesicles in cell bodies and dendrites; the localization in axons is also vesicular [4,6].

Two Hcrt/Ox G-protein-coupled receptors have been described. They show 64% molecular homology. The structure of Hcrt1R/OX_1R is similar to most peptidergic receptors, and shows higher affinity for Hcrt1/OxA than for Hcrt2/OxB [5,10]. Hcrt1R/OX_1R RNAm is mainly expressed in the ventromedial hypothalamic nucleus (HVM), though it is also abundant in the bed nucleus of the stria terminalis, the hippocampal formation (CA1, CA2), amygdala, tenia tecta, mesopontine reticular formation, RDo, median raphe nucleus and LC [10,12].

Hcrt2R/OX_2R binds Hcrt1/OxA and Hcrt2/OxB with similar affinity. Hcrt2R/OX_2R is present in the paraventricular nucleus of the hypothalamus, and also in the cerebral cortex, mainly in layer VI; it is also present in the olfactory tubercle, basal ganglia, hypothalamus, TMN, accumbens nucleus, BF, paraventricular and central medial thalamic nuclei, mesopontine

reticular formation and median raphe nucleus; Hcrt2R/OX_2R usually shows lower expression than Hcrt1R/OX_1R [10,12].

The main functions ascribed to Hcrt/Ox are [6]:

Feeding stimulation

Hcrt1/OxA, and to a lesser degree Hcrt2/OxB, stimulate food intake. Neurons containing Hcrt/Ox are glucose-sensitive [5]. Moreover, these neurons also have receptors for leptin [10] and STAT 3 (transcriptional factor activated by leptin) [13].

Neuroendocrine and cardiovascular regulation

Hcrt/Ox cells innervate the hypothalamic arcuate nucleus affecting its synaptic activity. This nucleus regulates adenohypophysis hormonal secretion and controls blood pressure [6,14]. Hcrt/Ox also modulate GH and prolactin plasma levels [15]. In addition, Hcrt/Ox receptors are abundant in other nuclei involved in vegetative functions, for instance Barrington's nucleus, which participates in micturition and colon motility [16].

The perifornical area and lateral hypothalamus (HL) are involved in cardiovascular responses associated to emotion. The perifornical region receives afferents from brain areas related to cardiovascular function, such as the ventromedial periaqueductal grey matter, RDo and LDT; Hcrt/Ox neurons also project to brain regions participating in cardiovascular function regulation, such as the ventrolateral medulla, lateral paragigantocellular nucleus, LC, periaqueductal region, parabrachial region and area postrema [6].

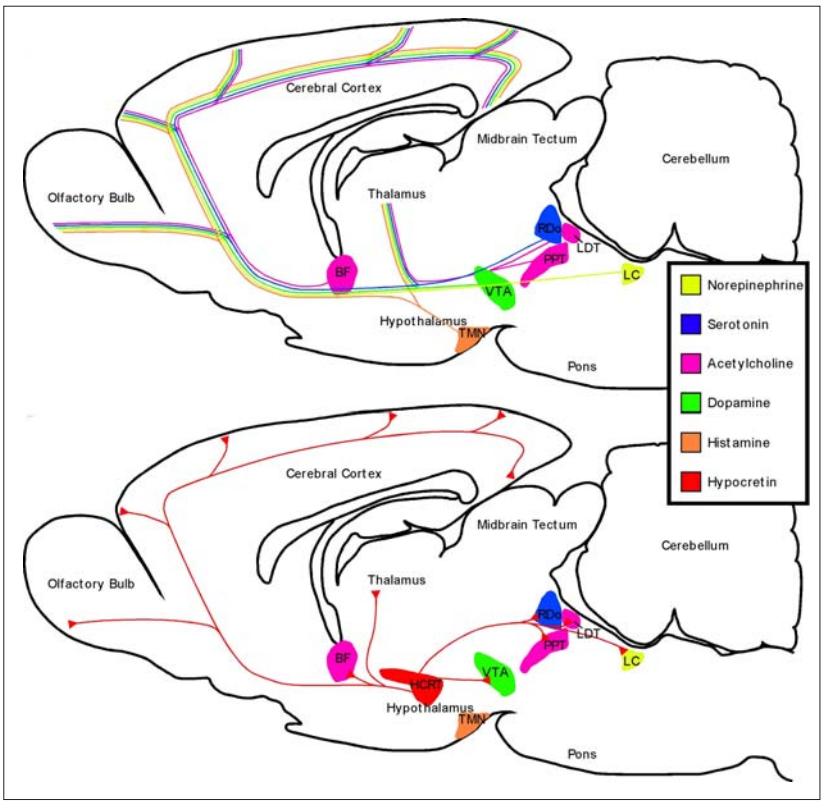

Figure 2. Sagittal schemes of the rat brain illustrating non-thalamic subcortical activating influences on the cerebral cortex. a) Neurochemically-specific neurons within the locus coeruleus (LC), dorsal raphe nucleus (RDo), laterodorsal tegmental nucleus (LDT), peduculopontine tegmental nucleus (PPT), ventral tegmental area (VTA), tuberomammilary nucleus (TMN) and basal forebrain (BF) send direct ascending axons to the cerebral cortex; b) Hypocretin/orexin (HCRT) hypothalamic neurons send axons to both the cerebral cortex and neurochemically-specific neuronal groups projecting to the cortex, such as LC, RDo, LDT, PPT, VTA, TMN and BF. Thus, HCRT neurons may activate the cerebral cortex both directly and indirectly through the switching on of subcortical wake-enhancing structures.

Sympathetic and parasympathetic system homeostatic regulation

LC shows intense Hcrt/Ox innervation as well as a remarkable level of Hcrt1R/OX_1R expression [6,13,16-18].

Thermoregulation

Many Hcrt/Ox-immunoreactive axons reach nuclei involved in body temperature regulation, such as the nucleus raphe magnus, HL and subcoeruleus nucleus. Furthermore, Hcrt/Ox neurons themselves are located in HPL, another structure implicated in this function [6].

Pain modulation

The presence of Hcrt/Ox immunoreactivity in the marginal zone of the spinal cord suggests that Hcrt/Ox could participate in nociceptive modulation [19]. Moreover, intracerebroventricular and intrathecal administration of Hcrt1/OxA and Hcrt2/OxB has antinociceptive effects in mice [20]. This regulation could also occur through the raphe nuclei, which present strong immunoreactivity for Hcrt/Ox and their receptors [6,16].

Sleep-wakefulness cycle regulation

See below.

SLEEP-WAKEFULNESS CYCLE

Hypothalamic involvement in wake and sleep states has been known since the classic descriptions by Von Economo in the beginning of the 20th century [21]. The posterior hypothalamus is a wakefulness-promoting region while the anterior hypothalamus enhances sleep [22].

Bremer was the first researcher who postulated that wakefulness is maintained by tonic impulses ascending from brainstem to prosencephalic structures [23]. Cortical activation during the wakefulness state responds to subcortical tonic impulses from the reticular formation and the posterior hypothalamus, which are fundamental structures for the generation of this state. They configure the reticular activating system described by Moruzzi et al [24]. In the 70's, Hobson et al [25] observed that some specific neuronal groups of the reticular formation, namely the aminergic neurons (mainly noradrenergic cells in LC and serotonergic cells in raphe nuclei), present an intense activity in wakefulness that progressively decreases during non-REM sleep to cease in REM sleep. The reciprocal interaction between aminergic and cholinergic neurons was suggested as the basis of sleep-wakefulness alternation by those authors at that time.

Nowadays, it is known that the arousal system activating the cerebral cortex includes excitatory neurons with diverse neuro-

chemical phenotypes (Fig. 2): glutamatergic tegmental neurons, noradrenergic LC neurons, serotonergic rostral raphe neurons, dopaminergic VTA neurons and histaminergic TMN neurons. The cholinergic neurons activating the cortex are mainly located in the basal forebrain (BF), but also in cholinergic nuclei of the mesopontine tegmentum (PPT and LDT). Neurons in all these nuclei containing well-known specific neurotransmitters may exert their excitatory actions on the cortex either directly or through their projections to the thalamus and activation of the thalamocortical systems [26,27].

The Hcrt/Ox neuropeptides are considered delicate modulators of all these wake-enhancing systems since hypothalamic Hcrt/Ox neurons project heavily to them. Normal wakefulness needs the concurrent and synergic actions of specific neurotransmission systems on the thalamus and cerebral cortex. The distribution of these neurotransmitters in distinct thalamic nuclei and individual cortical areas is not homogeneous, but different and specific to each transmitter and to each region, so that their roles in wakefulness should present different characteristics for each neurotransmission system [28,29].

Indirect pathway: tegmentum-thalamus-cortex

Tegmental nuclei (LC, RDo, VTA, TMN, LDT, PPT and BF) using the above-mentioned neurotransmitters project to the thalamus in individual patterns and facilitate cortical activation through facilitation of thalamocortical systems.

Direct pathway: tegmentum-cortex

Direct actions of each neurotransmitter on the cerebral cortex result in particular traits of the subsequent wakefulness; in other words, each neurotransmitter seems to be especially involved in definite aspects of wakefulness [28]:

- *Noradrenaline (NA):* has a phasic effect on cortical neurons, in that it enhances the selection of appropriate responses in a hyperstimulated environment, such as awakening, arousal, attention, awareness and learning [29-31].
- *Serotonin (5-HT):* exerts a tonic effect on cortical neurons and is important in the modulation of cortical neurons during phase changes or adjustment of different wakefulness levels [29,31].
- *Dopamine (DA):* has a more uniform release than NA or 5-HT during the SWC. It modulates cortico-cortical circuits and integrative processes [29,31].
- *Histamine (HA):* is released specifically during wakefulness in the cortex in a tonic manner and it is involved in maintaining the awakened state during novel circumstances [31]. It also regulates motor activity and inhibits REM sleep [32,33].
- *Acetylcholine (Ach):* enhances both waking and REM sleep, resulting in the generation of fast rhythms in the cortical EEG [29, 31,34].

HCRT/OX SYSTEM AND SLEEP

The distribution of Hcrt/Ox neurons projections from the hypothalamus throughout the central nervous system of the rat has been studied by immunohistochemical methods. The axons of the Hcrt/Ox neurons present a great variability in thickness and have a high number of varicosities [6]. It is surprising that such a limited number of Hcrt/Ox neurons have such a widespread projection system (Fig. 1). In summary form:

- *Projections to the brainstem:*
  a) Dorsal pathway: fibers passing through the central gray matter arrive at the inferior colliculus, LC, RDo and LDT. Some other fibers cross the dorsal tegmental area and reach the PPT, parabrachial region and nucleus subcoeruleus.
  b) Ventral pathway: projections to the VTA, substantia nigra compacta, raphe nucleus, caudal and oral pontine reticular formation and nucleus subcoeruleus.
- *Projections to the diencephalon and telencephalon:*
  a) Hypothalamus: mainly to the tuberomammillary nucleus and arcuate.
  b) Thalamus: paraventricular nucleus and central medial nucleus.
  c) BF.
  d) Cerebral cortex.

These projections suggest that Hcrt/Ox neurons could participate in cognitive, autonomic, motivational and emotional functions [5,6,8]. As regards the regulation of SWC, Hcrt/Ox neurons densely innervate structures belonging to the ascending reticular activating system responsible for wakefulness (LC, RDo, TMN, VTA, PPT/LDT y BF) [6], and these structures express Hcrt/Ox receptors [12,16]. Therefore, Hcrt/Ox neurons could help maintain the wakefulness state via activation of the ascending reticular activating system.

Aminergic and cholinergic neurons have been classified in two different categories depending on their activity during REM sleep (Table):

- *REM off neurons:* these neurons contain NA in the LC, 5-HT in the rostral raphe or His in the TMN. They are active during wakefulness and stop firing during REM sleep [25]. Hcrt/Ox excite these aminergic neurons [17,18,33,35].
- *REM on neurons:* they are cholinergic neurons located in the PPT/LDT and the BF [34].

It seems that Hcrt/Ox neuropeptides have excitatory actions, showing the highest secretion peak at the end of wakefulness in both nocturnal and diurnal species [36]; for that reason the Hcrt/Ox are thought to stabilize the wakefulness state, so their absence could explain the alterations in sleep states occurring in narcoleptic patients [4]. The involvement of Hcrt/Ox in motor control and the regulation of postural muscle tone could account for the presence of cataplexy in narcoleptics [36,37].

In order to explain Hcrt/Ox actions in SWC, Kilduff and Peyron proposed a theoretical model in which Hcrt/Ox neurons were assumed to be REM on/wake on neurons [13]. According to these authors, during wakefulness Hcrt/Ox neurons excite monoaminergic neurons, which in turn provoke the characteristic cortical desynchronization of the waking state. During non-REM sleep, the activity of Hcrt/Ox neurons is reduced by inhibitory inputs from GABAergic neurons of the anterior hypothalamus. This would lead to decreased neuronal activity in the LC, RDo, TMN and VTA, and subsequent synchronization of the thalamocortical circuits. Hcrt/Ox neurons are active mainly during wakefulness according to the hypothesis of Kilduff et al, but also during REM sleep [13,38], during which cortical activation is similar to that during wakefulness; these authors propose that monoaminergic neurons are not available for excitation during REM sleep, since they would be in a silent resting condition. LDT/PPT neurons (REM on group) would be disinhibited, increasing cortical de-

synchronization [13,31]. However, later studies have reported reduced activity of Hcrt/Ox neurons during REM sleep [3].

Saper et al [2] have proposed another model for Hcrt/Ox regulation of SWC (the 'flip-flop' model) that implies a bi-directional connection between the ventrolateral preoptic area (VLPO) of the anterior hypothalamus and aminergic nuclei (LC, RDo, TMN). According to this model, VLPO and LC/RDo/TMN exert antagonistic effects in the wakefulness state in such a way that VLPO would inhibit wakefulness and LC/RDo/TMN would enhance it. Both systems inhibit each other reciprocally, displaying a bi-stable balanced state (wakefulness-LC/RDo/TMN or sleep-VLPO). Hcrt/Ox might stabilize changes between the two states by acting on both. Thus, Hcrt/Ox neurons would be REM off/wake on in this model [2].

Table. Firing activity of different neurochemically-specific neuronal groups during wakefulness and REM sleep states.

	Wakefulness	REM
Hcrt/Ox	++++	–
RDo (5-HT), LC (NA), TMN (His)	++++	–
VTA (DA)	+++	–
BF (ACh)	+++	+++
LDT, PPT (ACh)	++	++++

++++: very intense; +++: intense; ++: moderate; –: silent.

Hcrt/Ox and LC

LC neurons are known to be the origin of an extensive noradrenergic innervation of the cerebral cortex involved in attention, learning, memory and the sleep-wakefulness cycle (Figs. 1 and 2) [39,40]. Selective activation of LC is sufficient in itself, though not indispensable, to produce EEG cortical activation [30].

On the other hand, LC neurons present many Hcrt/Ox receptors, mainly of the type Hcrt1R/OX_1R [12,16]. Hcrt/Ox administration in the rat LC produces and increases wakefulness and suppresses REM sleep [41]. Moreover electrophysiological recordings have shown that Hcrt/Ox application increases the discharge rate of NA LC neurons [17,18].

The cellular interactions established between Hcrt/Ox axons from the hypothalamus and the local NA neurons in the rat LC have been assessed; most of the observed synapses were asymmetric, being either axo-dendritic or axo-somatic [17]. The data obtained by measuring the extracellular action potential of LC neurons in basal conditions and after Hcrt/Ox application, suggest that Hcrt1/OxA, and also Hcrt2/OxB to a lesser degree, has an essential role in the intrinsic modulation of these neurons, increasing their firing rate through activation of Hcrt1R/OX_1R [18]. All these observations demonstrate that the Hcrt/Ox system excites NA LC neurons.

Hcrt/Ox and RDo

The dorsal raphe nucleus is located in the ventromedial portion of the pediaqueductal grey matter, at the mesopontine junction and projects abundantly to the cerebral cortex (Figs. 1 and 2) [42]. It is one of the CNS structures to contain the most serotonergic neurons. Serotonin can have excitatory and inhibitory effects on the cortex, since it acts on both pyramidal neurons and GABA interneurons [43]. The mechanisms of action are unknown at the present time, although the results obtained after low frequency stimulation of the raphe nuclei suggest that physiological levels of 5-HT may inhibit pyramidal neurons [44]. Hcrt/Ox neurons intensely innervate the RDo [6,45], suggesting that RDo could be a major structure through which Hcrt/Ox would facilitate wakefulness [46].

Both Hcrt1R/OX_1R and Hcrt2R/OX_2R are present in the RDo [5,12,16]. It is accepted that serotonergic neurons are activated mainly through Hcrt2R/OX_2R; this activation may be augmented by Hcrt1R/OX_1R activation, which would increase the firing rate of these neurons.

Continuous application of Hcrt/Ox in the RDo produces enduring effects, as also occurs in LC [47] and in the substantia nigra [48]; these structures show no adaptation to Hcrt/Ox activating effects. On the contrary, the excitatory action of Hcrt/Ox in the LDT decreases with time.

Electron microscope experiments indicate that Hcrt/Ox are located mostly in large dense core vesicles in axon terminals of the RDo. These terminals make preferably asymmetric synapses in axo-dendritic contacts and symmetric synapses in axo-somatic contacts [49].

Hcrt/Ox and TMN

The tuberomammillary nucleus of the posterolateral hypothalamus is the origin of the histaminergic innervation to the cerebral cortex; histaminergic neurons within this nucleus discharge vigorously during wakefulness, decrease their activity at the beginning of sleep and are inactive through the REM phase [31,50,51]. Hcrt/Ox neurons intensely project to the TMN [6], and Hcrt/Ox depolarize TMN histaminergic cells and increase their discharge rate [32,33].

Experiments performed in knockout mice indicate that the arousing effect of Hcrt/Ox depends to a great extent on the activation of histaminergic cells [52]. Histamine levels are very low in the cerebral cortex of the narcoleptic dogs [53]. Most probably GABAergic projections from the anterior hypothalamus to the TMN participate in the inhibition of histaminergic neurons during sleep [54].

Hcrt/Ox and VTA

The ventral tegmental area of the midbrain contains many dopaminergic neurons that project to cortical and limbic targets. It receives a dense Hcrt/Ox-immunoreactive input [6] and expresses Hcrt/Ox receptors [55]. Even though Hcrt/Ox application in the VTA increases dopamine release in the cerebral cortex [56], VTA neuronal responses to Hcrt/Ox are very heterogeneous: increase of discharge in bursts, increased discharge rates or an absence of effect have all been described, in both dopaminergic and non-dopaminergic neurons [57]. Hcrt/Ox infusion in the VTA enhances wakefulness and cortical EEG activation [56].

Hcrt/Ox and BF-PPT/LDT

Hcrt/Ox neurons project to the basal forebrain [6], where their axons establish asymmetric synapses with local cholinergic neurons [58], strongly activating them [59]. Moreover, Hcrt/Ox application in the BF enhances wakefulness [60] and increases cortical acetylcholine release [61]. Hcrt/Ox also activates the mesopontine cholinergic neurons (LDT/PPT) [62], which are the main origin for the cholinergic input to the thalamus and participate actively in the cortical activation appearing in wake-

fulness and REM sleep [63]. Hcrt/Ox injection in LDT increases wakefulness and decreases REM sleep [64].

Hcrt/Ox and vRPO

The vRPO is in the most sensitive region within the oral pontine tegmentum for pharmacologic induction of REM sleep [27]. It is targeted by hypothalamic Hcrt/Ox neurons [7]. Hcrt/Ox microinjections in the vRPO produce a decrease in REM sleep that is associated or not (depending on the dosage) with increased wakefulness [65]. In addition, Hcrt/Ox inhibit the vRPO neuronal activity; this effect is very probably dependent on GABA receptor activation, since it can be locally blocked with bicuculine ($GABA_A$ antagonist) [7]. During wakefulness Hcrt/Ox neurons probably inhibit REM sleep generation mechanisms in the vRPO. We propose that this inhibition periodically disappears in normal conditions with the appearance of REM sleep, and therefore, Hcrt/Ox neurons could be a type of REM off/wake on neuron. In narcolepsy, the absence of this inhibition as the result of insufficient Hcrt/Ox actions could originate the presence of sudden bouts of REM sleep during wakefulness and loss of SWC rhythm. The typical REM sleep attacks (and wakefulness inhibition) of narcoleptics could therefore be due to REM sleep pontine generator (vRPO) disinhibition. That is to say Hcrt/Ox neuron activation would provoke wakefulness while simultaneously inhibiting REM sleep generation.

HYPOCRETIN/OREXIN AND NARCOLEPSY

The main clinical signs of narcolepsy are excessive daytime sleepiness and premature initiation of REM sleep (shortened REM latency), sometimes directly from wakefulness without a preceding slow wave sleep period. Narcoleptic patients also show very low or undetectable levels of Hcrt/Ox in CSF [9]. Furthermore, there are animal models of narcolepsy consisting in modifications of hypocretin/orexin receptors [66] or absence of the peptides [67]. For this reason, nowadays it is assumed that narcolepsy is the direct consequence of Hcrt/Ox neuron degeneration, and therefore indicates widespread hypocretinergic/orexinergic hypofunction. This anomaly would modify the normal transition between the different states of consciousness and cortical activation.

The SWC is disorganized in narcoleptic patients. In addition to their excessive daytime sleepiness and the REM sleep alteration, narcoleptics may present loss of consciousness and, less frequently, cataplexy (sudden muscle tone loss despite maintained consciousness, generally caused by a sensory stimulus or strong emotion). They may also have hypnagogic/hypnopompic hallucinations and sleep paralysis (temporary paralysis shortly after waking or before falling asleep). All these symptoms seem to be secondary to an altered regulation of sleep states and a disorganization of nocturnal sleep. The prevalence of narcolepsy is approximately 1:2000-1:4000, the first symptoms usually appear in teenagers and there are no gender differences [36,68].

Excessive daytime sleepiness and cataplexy are the key symptoms for narcolepsy diagnosis; the rest of the symptoms are less frequent and can appear in other diseases or even in healthy subjects. Narcoleptic patients usually present only some of the symptoms, and the complete clinical picture is seldom observed in a single individual. Narcolepsy symptoms are ascribed to a failure of REM sleep regulation. Thus, cataplexy and sleep paralysis could be an intrusion into wakefulness of the typical REM sleep muscle atonia. Hallucinations could be interpreted as the expression during wakefulness of oniric phenomena characteristic of REM sleep. Total sleep times are similar in narcoleptics and healthy subjects, but narcoleptics have difficulty in maintaining long periods of either wakefulness or sleep [36].

There are different reasons to link Hcrt/Ox and narcolepsy. Narcoleptic patients have fewer Hcrt/Ox neurons in the HPL than control subjects [69,70], and their CSF shows lower or untraceable Hcrt/Ox levels [9,71]. Moreover, gliosis has been reported in the perifornical area in some narcoleptic patients [69,70]. All these observations, together with the known association of narcolepsy with specific antigens of the major histocompatibility system (HLA), suggest that narcolepsy could be an autoimmune/degenerative disease. The astrocytic marker GFAP (glial fibrillary acidic protein) seems to be present in just some of these patients, although that is not definitive since the analyzed tissue had been stored for a long time and could have lost immunoreactivity [69]. In addition, hereditary canine narcolepsy is due to a mutation in *Hcrt2R/OX$_2$R* [66] and, in mice, deletion of the *Hcrt/Ox* gene produces narcoleptic symptoms [67]. Although Hcrt/Ox neuron degeneration is the most accepted hypothesis for human narcolepsy, other possible causes include defects in the synthesis of Hcrt/Ox or their receptors.

Even though the heredity of human narcolepsy is low, it is clear that genetic components exist in its pathogenesis; several theories have been proposed regarding genetic factors in narcolepsy. The most accepted hypotheses are:

- *HLA II allele mutation in leucocytes (DQB1*0602).* This haplotype is present in approximately 30% of the population, and the mutation has been observed in more than 90% of the narcoleptics exhibiting cataplexy; thus, narcolepsy is one of the diseases showing a high association with a specific HLA allele. This is why narcolepsy is thought to be a possibly autoimmune disease [36,68,71,72].
- Hcrt/Ox *gene.* A mutation in *Hcrt2/OxB* gene with a recessive autosomal heredity causing narcolepsy has been detected in narcoleptic dogs. The *Hcrt2/OxB* mutation is involved in cataplexy and REM sleep alteration [53,66,73,74]. At this time only a *preproHcrt/preproOx* gene mutation has been described in humans [70].

Nevertheless, the results obtained in animal models do not exactly correlate with the degeneration hypothesis; for example, Hcrt2/OxB –/– knock-out (KO) dogs present gliosis in structures outside the hypothalamus [73] and Hcrt/Ox –/– KO mice do not exhibit a general disorganization of SWC [67,75,76].

The current available data indicate that rather than a unique nosological unit, human narcolepsy is a syndromic group that most likely includes disorders with different causes but similar signs. Undoubtedly, the diagnosis and treatment of narcolepsy will be improved once we identify the different partial components of this syndrome.

At the present time, Hcrt/Ox are considered to be neuromodulators that enhance the waking state through their activity in LC, RDo, LDT, PPT, VTA and TMN, and also inhibit REM sleep by acting on the vRPO [2,7]. Impairment of the Hcrt/Ox neuron projection system or actions would provoke, on one hand, hypoactivity of the ascending activating systems, decreasing cerebral cortex stimulation, and, on the other hand, disinhibition of the vRPO and a triggering of REM sleep. This hypothesis could explain the great number of transitions between wake-

fulness and sleep, REM sleep fragmentation and hypersomnia present in narcoleptic patients [77].

Theta activity in the EEG and atonia are shared features in cataplexy and REM sleep. However, experiments performed in Hcrt/Ox –/– KO mice [75,76] suggest that RDo and TMN neurons discharge during cataplexy but are silent in REM sleep. These mice had a slight REM alteration with shortened latency but no significant changes were observed in their wakefulness or non-REM sleep periods. Overall, the sleep-wakefulness states had the usual characteristics, although the mean duration of the episodes was very short. Some authors consider cataplexy to be either a fragmentary REM manifestation or else a transition state between wakefulness and REM sleep.

The following are some neurobiological models proposed to explain sleepiness in narcoleptics:

– *Deficient hypocretinergic/orexinergic system*. Hcrt/Ox neurons excite structures involved in wakefulness generation: aminergic (LC, RDo, VTA) and cholinergic (PPT/LDT) neurons in the brainstem, histaminergic neurons in the hypothalamus (TMN) and cholinergic neurons in the BF [6,13,18, 32,49,57,59,62]. Decreased activity of these neurons facilitates thalamocortical circuits synchronization and sleep emergence [63,78]. Idiopathic hypersomnolence has been reported in association with reduced Hcrt1/OxA levels in CSF [36].
– *Loss of circadian control.* The suprachiasmatic nucleus plays an essential role in SWC regulation. It is known that Hcrt/Ox neurons and the suprachiasmatic nucleus are interconnected [79]. Narcoleptic patients have at least two REM sleep breakouts during wakefulness; these episodes could be a consequence of a decrease in the strength of circadian regulation [80].

CONCLUSIONS

Hcrt/Ox are peptides exclusively synthesized by a small group of neurons in the perifornical area of HPL; these neurons are the origin of an extensive and divergent projection system innervating numerous structures of the CNS.

These neuropeptides are involved in the regulation of many organic functions, such as SWC, feeding, thermoregulation and neuroendocrine and cardiovascular control.

Hcrt/Ox neurons enhance wakefulness through their excitatory projections to aminergic (RDo, LC, VTA, TMN) and cholinergic (BF, PPT/LDT) structures implicated in the generation of that behavioural state, and they inhibit REM sleep through their actions in vRPO.

Narcoleptic patients show minimal or undetectable levels of Hcrt/Ox in CSF, a reduced number of Hcrt/Ox neurons, and partial gliosis in the HPL. It is currently thought that these observations may be secondary to Hcrt/Ox neuron degeneration. Moreover, more than 90% of narcoleptic-cataplectic individuals have a specific HLA II allele mutation, indicating that this degeneration would probably have an inherited autoimmune cause.

Experimental studies in animals indicate that a mutation in either Hcrt/Ox peptide or Hcrt/OX receptors partially reproduces human narcolepsy signs. However, until now only a mutation of the *preproHcrt/preproOx* gene has been reported in narcoleptic human patients.

The discovery of the pathogenic mechanisms that underlie the loss of Hcrt/Ox neurons in humans will constitute a crucial boost for narcolepsy research in the future. That information is essential for the prevention and treatment of the disease.

REFERENCES

1. Bayer L, Serafin M, Eggermann E, Saint-Mieux B, Machard D, Jones BE, Mühlethaler M. Exclusive postsynaptic action of hypocretin-orexin on sublayer 6b cortical neurons. J Neurosci 2004; 24: 6760-4.
2. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 2001; 24: 726-31.
3. Lee GM, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J Neurosci 2005; 25: 6716-20.
4. De Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998; 95: 322-7.
5. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G-protein. Coupled receptors that regulate feeding behaviour. Cell 1998; 92: 573-85.
6. Peyron C, Tighe DK, Van den Pol AN, De Lecea L, Heller HC, Sutcliffe JG, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18: 9996-10015.
7. Núñez A, Moreno-Balandrán ME, Rodrigo-Angulo ML, Garzón M, De Andrés I. Relationship between the perifornical hypothalamic area and oral pontine reticular nucleus in the rat. Possible implication of the hypocretinergic projection in the control of rapid eye movement sleep. Eur J Neurosci 2006; 24: 2834-42.
8. Chen CT, Dun SL, Kwok EH, Dun NJ, Chang JK. Orexin A-like immunoreactivity in the rat brain. Neurosci Lett 1999; 260: 161-4.
9. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355: 39-40.
10. Sakurai T. Orexins and orexin receptors: implication in feeding behaviour. Regul Pept 1999; 85: 25-30.
11. Johren O, Neidert SJ, Kummer M, Dendorfer A, Dominiak P. Prepro-orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female rats. Endocrinology 2001; 142: 3324-31.
12. Trivedi P, Yu H, MacNeil DJ, Van der Ploeg LH, Guan XM. Distribution of orexin receptor mRNA in the rat brain. FEBS Lett 1998; 438: 71-5.
13. Kilduff TS, Peyron C. The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. Trends Neurosci 2000; 23: 359-65.
14. Van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB. Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. J Neurosci 1998; 18: 7962-71.
15. De Lecea L, Sutcliffe JG, Fabre V. Hypocretins/orexins as integrators of physiological information: lessons from mutant animals. Neuropeptides 2002; 36: 85-95.
16. Greco MA, Shiromani PJ. Hypocretin receptor protein and mRNA expression in the dorsolateral pons of rats. Mol Brain Res 2001; 88: 176-82.
17. Horvath TL, Peyron C, Diano S, Ivanov A, Aston-Jones G, Kilduff TS, et al. Hypocretin (orexin) activation and synaptic innervations of the locus coeruleus noradrenergic system. J Comp Neurol 1999; 415: 145-59.
18. Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, et al. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci U S A 1999; 96: 10911-6.
19. Van den Pol AN. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. J Neurosci 1999; 19: 3171-82.
20. Mobarakeh JI, Takahashi K, Saturada S, Nishino S, Watanabe H, Kato M, et al. Enhanced antinociception by intracerebroventricularly and intrathecally-administered orexin A and B (hypocretin-1 and -2) in mice. Peptides 2005; 26: 767-77.
21. Von Economo C. Sleep as a problem of localization. J Nerv Ment Dis 1930; 71: 249-59.
22. Nauta WGH. Hypothalamic regulation of sleep in rat. An experimental study. J Neurophysiol 1946; 9: 285-316.
23. Bremer F. Cervau isolé et physiologie du sommeil. C R Soc Biol 1935; 118: 1235-41.
24. Moruzzi G, Magoun HW. Brainstem reticular formation an activation of the EEG. EEG Clin Neurophysiol 1949; 1: 455-73.
25. Hobson JA, McCarley RW, Wyzinski PW. Sleep cycle oscillation: reciprocal discharge by to brainstem neuronal groups. Science 1975; 189: 55-8.
26. Reinoso-Suárez F, Llamas A. Conexiones aferentes a la corteza frontal desde tegmento pontomesencefálico (locus coeruleus, rafe, sustancia negra) en la rata. An Anat 1975; 24: 337-50.
27. Reinoso-Suárez F, De Andrés I, Rodrigo-Angulo ML, Garzón M.

Brain structures and mechanisms involved in the generation of REM sleep. Sleep Med Rev 2001; 5: 63-7.
28. Reinoso-Suárez F. Neurobiología del despertar y la vigilia. An R Acad Nac Med 1997; 114: 249-64.
29. Reinoso-Suárez F. Neurobiología del sueño. Rev Med Univ Navarra 2005; 49: 10-7.
30. Berridge CW, Foote SL. Effects of locus coeruleus activation on electroencephalographic activity in neocortex and hippocampus. J Neurosci 1991; 11: 3135-45.
31. Mignot E, Taheri S, Nishino S. Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci Suppl 2002; 5: 1071-5.
32. Bayer L, Eggermann E, Serafin M, Saint-Mleux B, Machard D, Jones B, et al. Orexins (hypocretins) directly excite tuberomammillary neurons. Eur J Neurosci 2001; 14: 1571-5.
33. Eriksson KS, Sergeeva O, Brown RE, Haas HL. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J Neurosci 2001; 21: 9273-9.
34. Jones BE. Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. Prog Brain Res 2004; 145: 157-69.
35. Brown RE, Sergeeva O, Eriksson KS, Haas HL. Orexin A excites serotonergic neurons in the dorsal raphe nucleus of the rat. Neuropharmacology 2001; 40: 457-9.
36. Martínez-Rodríguez JE, Santamaría J. Narcolepsia e hipersomnia idiopática. Rev Med Univ Navarra 2005; 49: 35-40.
37. Salín-Pascual RJ. Hipocretinas y adenosina en la regulación del sueño. Rev Neurol 2004; 39: 354-8.
38. Kiyashchenko LI, Mileykovskiy BY, Maidment N, Lam HA, Wu MF, John J, et al. Release of hypocretin (orexin) during waking and sleep states. J Neurosci 2002; 22: 5282-6.
39. Singewald N, Philippu A. Release of neurotransmitters in the locus coeruleus. Prog Neurobiol 1998; 56: 237-67.
40. Loughlin SE, Foote SL, Grzanna R. Efferent projections of nucleus locus coeruleus: morphologic subpopulations have different efferent targets. Neuroscience 1986; 18: 307-19.
41. Bourgin P, Huitron-Resendiz S, Spier AD, Fabre V, Morte B, Criado JR, et al. Hypocretin-1 modulates rapid eye movement sleep through activation of LC neurons. J Neurosci 2000; 20: 7760-5.
42. Vertes. A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. J Comp Neurol 1991; 313: 643-68.
43. Puig MV, Artigas F, Celada P. Modulation of activity of pyramidal neurons in rat prefrontal cortex by raphe stimulation in vivo: involvement of serotonin and GABA. Cereb Cortex 2005; 15: 1-14.
44. Puig MV, Celada P, Artigas F. Control serotoninérgico de la corteza prefrontal. Rev Neurol 2004; 39: 539-47.
45. Lee HS, Park SH, Song WC, Waterhouse BD. Retrograde study of hypocretin-1 (orexin-A) projections to subdivisions of the dorsal raphe nucleus in the rat. Brain Res 2005; 1059: 35-45.
46. Tao R, Ma Z, McKenna JT, Thakkar MM, Winston S, Strecker RE, et al. Differential effect of orexins (hypocretins) on serotonin release in the dorsal and median raphe nuclei of freely behaving rats. Neuroscience 2006; 141: 1101-5.
47. Siegel JM. The neurotransmitters of sleep. J Clin Psychiatry 2004; 65 (Suppl 16): 4-7.
48. Korotkova TM, Eriksson KS, Haas HL, Brown RE. Selective excitation of GABAergic neurons in the sustantia nigra of the rat by orexin/hypocretin in vitro. Regul Pept 2002; 104: 83-9.
49. Wang QP, Guan JL, Matsuoka T, Hirayana Y, Shioda S. Electron microscopic examination of the orexin immunoreactivity in the dorsal raphe nucleus. Peptides 2003; 24: 925-30.
50. Vanni-Mercier G, Gigout S, Debilly G, Lin JS. Waking selective neurons in the posterior hypothalamus and their response to histamine H3-receptor ligands: an electrophysiological study in freely moving cats. Behav Brain Res 2003; 144: 227-41.
51. Ko EM, Estabrooke IV, McCarthy M, Scammell TE. Wake-related activity of tuberomammillary neurons in rats. Brain Res 2003; 992: 220-6.
52. Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, et al. Arousal effect of orexin A de pends on activation of the histaminergic system. Proc Natl Acad Sci U S A 2001; 98: 9965-70.
53. Nishino S, Fujiki N, Ripley B, Sakurai E, Kato M, Watanabe T, et al. Decreased brain histamine content in hypocretin/orexin receptor-2 mutated narcoleptic dogs. Neurosci Lett 2001; 313: 125-8.
54. Sherin JE, Elmquist JK, Torrealba F, Saper CB. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. J Neurosci 1998; 18: 4705-21.
55. Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, et al. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 2001; 435: 6-25.
56. Vittoz NM, Berridge CW. Hypocretin/orexin selectively increases dopamine efflux within the prefrontal cortex: involvement of the ventral tegmental area. Neuropsychopharmacology 2006; 31: 384-95.
57. Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci 2003; 23: 7-11.
58. Wu M, Zaborszky L, Hajszan T, Van den Pol AN, Alreja M. Hypocretin/orexin innervation and excitation of identified septohippocampal cholinergic neurons. J Neurosci 2004; 24: 3527-36.
59. Eggermann E, Serafin M, Bayer L, Machard D, Saint-Mleux B, Jones BE, et al. Orexins/hypocretins excite basal forebrain cholinergic neurones. Neuroscience 2001; 108: 177-81.
60. Thakkar MM, Ramesh V, Strecker RE, McCarley RW. Microdialysis perfusion of orexin-A in the basal forebrain increases wakefulness in freely behaving rats. Arch Ital Biol 2001; 139: 313-28.
61. Fadel J, Pasumarthi R, Reznikov LR. Stimulation of cortical acetylcholine release by orexin A. Neuroscience 2005; 130: 541-7.
62. Burlet S, Tyler CJ, Leonard CS. Direct and indirect excitation of laterodorsal tegmental neurons by hypocretin/orexin peptides: implications for wakefulness and narcolepsy. J Neurosci 2002; 22: 2862-72.
63. Steriade M, Datta S, Pare D, Oakson G, Curro Dossi RC. Neuronal activities in brain-stem cholinergic nuclei related to tonic activation processes in thalamocortical systems. J Neurosci 1990; 10: 2541-59.
64. Xi MC, Morales FR, Chase MH. Effects on sleep and wakefulness of the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. Brain Res 2001; 901: 259-64.
65. Moreno-Balandrán ME, Robayo OF, Garzón M, De Andrés I. Efectos de la orexina A en las fases del ciclo vigilia-sueño administrada a regiones colinoceptivas del tegmento pontino. Vigilia-Sueño 2004; 16: 91.
66. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98: 365-76.
67. Chemelli RM, Willie JT, Sinton C, Elmquist J, Scammell TS, Lee C, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999; 98: 437-51.
68. Van den Pol AN. Narcolepsy: neurodegenerative disease of the hypocretin system? Neuron 2000; 27: 415-8.
69. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27: 469-74.
70. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6: 991-7.
71. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnostic of narcolepsy and other hypersomnias. Arch Neurol 2002; 59: 1553-62.
72. Tafti M, Nishino S, Aldrich MS, Liao W, Dement WC, Mignot E. Major histocompatibility class II molecules in the CNS: increased microglial expression at the onset of narcolepsy in canine model. J Neurosci 1996; 16: 4588-95.
73. John J, Wu MF, Maidment NT, Lam HA, Boehmer LN, Patton M, et al. Developmental changes in CSF hypocretin-1 (orexin-A) levels in normal and genetically narcoleptic Doberman pinschers. J Physiol 2004; 560: 587-92.
74. Siegel JM, Nienhuis R, Gulyani S, Ouyang S, Wu MF, Mignot E, et al. Neuronal degeneration in canine narcolepsy. J Neurosci 1999; 19: 248-57.
75. Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, et al. Distinct narcolepsy syndromes in orexin receptor-2 and orexin null mice: molecular genetic dissection of non-REM and REM sleep regulatory processes. Neuron 2003; 38: 715-30.
76. Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scalmmell TE. Behavioral state instability in orexin knockout mice. J Neurosci 2004; 24: 6291-300.
77. Soffin EM, Gill CH, Brough SJ, Jerman JC, Davies C. Pharmacological characterization of the orexin receptor subtype mediating postsynaptic excitation in the rat dorsal raphe nucleus. Neuropharmacology 2004; 46: 1168-76.
78. McCormick DA. Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. Prog Neurobiol 1992; 39: 337-88.
79. Abrahamson EE, Leak RK, Moore RY. The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. NeuroReport 2001; 12: 435-40.
80. Broughton R, Krupa S, Boucher B, Rivers M, Mullington J. Impaired circadian waking arousal in narcolepsy-cataplexy. Sleep Res Online 1998; 1: 159-65.

EL SISTEMA DE NEUROTRANSMISIÓN HIPOCRETINÉRGICO/OREXINÉRGICO EN LA REGULACIÓN DE LOS ESTADOS DE VIGILIA Y SUEÑO

Resumen. Introducción. *Las hipocretinas/orexinas son neuropéptidos sintetizados por un pequeño grupo neuronal localizado en el hipotálamo posterolateral. Desde el momento de su descubrimiento se relacionaron, entre otras funciones, con el ciclo vigilia-sueño. Concretamente, el sistema hipocretinérgico/orexinérgico muestra una gran actividad durante la vigilia; además, la deficiencia total o parcial de estos péptidos o de sus receptores se asocia al síndrome de narcolepsia-cataplejía, que cursa con un trastorno generalizado del ciclo vigilia-sueño.* Desarrollo. *Las neuronas hipocretinérgicas/orexinérgicas: a) activan directamente la corteza cerebral; b) activan grupos neuronales noradrenérgicos, serotoninérgicos, dopaminérgicos, colinérgicos e histaminérgicos que constituyen parte del sistema reticular ascendente de activación, y a través de este sistema, también pueden indirectamente producir activación cortical y aumento del estado de vigilia; y c) inhiben la generación de sueño REM en el tegmento pontino ventral. Durante el sueño, las neuronas hipocretinérgicas/orexinérgicas disminuyen su actividad y, en consecuencia, la de estos núcleos aminérgicos y colinérgicos del sistema reticular activador ascendente, favoreciendo una disminución en la activación cortical y la liberación de la génesis de sueño REM en el tegmento pontino ventral.* Conclusiones. *Las hipocretinas/orexinas regulan el mantenimiento de la vigilia y la activación del electroencefalograma, en parte a través de la inervación de neuronas reticulares de proyección cortical, y suprimen la aparición de sueño REM mediante la inhibición del tegmento pontino ventral. La hipoactividad de este sistema en la narcolepsia explicaría la desorganización y fragmentación del sueño, así como la intrusión de episodios de sueño REM en la vigilia. [REV NEUROL 2007; 45: 482-90]*

Palabras clave. *Hipocretina. Hipotálamo. Narcolepsia. Orexina. REM. Sueño. Vigilia.*