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REVIEW

HYPOCRETINS: THE TIMING OF SLEEP AND WAKING

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The appropriate time and place for sleep and waking are important factors for survival. Sleep and waking, rest and activity, flight and fight, feeding, and reproduction are all organized in relation to the day and night. A biological clock, the suprachiasmatic nucleus (SCN), synchronized by photic influences and other environmental cues, provides an endogenous timing signal that entrains circadian body rhythms and is complemented by a homeostatic sleep pressure factor. Cholinergic, catecholaminergic, serotonergic, and histaminergic nuclei control wakefulness and mutually interact with the SCN as well as sleep- and wake-promoting neurons in the hypothalamus to form a bistable switch that controlls the timing of behavioral state transitions. Hypocretin neurons integrate circadian-photic and nutritional-metabolic influences and act as a conductor in the aminergic orchestra. Their loss causes narcolepsy, a disease conferring the inability to separate sleep and waking. Their role in appetitive behavior, stress, and memory functions is important to our understanding of addiction and compulsion.

Keywords Hypocretins, Sleep, Orexin A and B, Circadian sleep-wake rhythm, Amines, Narcolepsy

INTRODUCTION

Hypocretins 1 and 2 (orexin A and B) were almost simultaneously discovered by two independent groups using reverse genetics (De Lecea et al., 1998) and pharmacology (Sakurai, 2003). The two peptides are derived from a common pre-pro-hypocretin gene, and both act with differential affinity on two G-protein-coupled receptors (Hcrt1/2R and OX1/2R, respectively). Hypocretin receptors are expressed in numerous targets throughout, and even outside, the nervous system.

Homologies with members of the secretin family of peptides and the almost exclusive expression in a small group of neurons located caudal to and in the perifornical area of the lateral hypothalamus prompted one the group of investigators to call them "hypothalamic secretins" (De Lecea et al., 1998), and evidence for a role in food intake and

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energy administration led to a second name, "orexins" (from Greek: orexis, meaning appetite) (Sakurai, 2003). Soon after their discovery, another important function of hypocretins became apparent. *i.e.*, the regulation of sleep-wake states and their absence in the sleep disorder narcolepsy-cataplexy. More recently, evidence for further functions ultimately subserving whole body homoeostasis and survival has accumulated (Mignot et al., 2002; Selbach et al., 2004; Siegel, 2004; Steidl et al., 2004; Paneda et al., 2005).

HYPOCRETIN NEURONS

Hypocretin neurons have widespread projections with an emphasis on infralimbic sensory and autonomic representations (Date et al., 1999; Krout et al., 2005), which form the major output centers for homeostatic and circadian signals to peripheral organs. Hypocretin neurons fire spontaneously during active waking, when muscle tone is high in association with movement (Mileykovskiy et al., 2005; Lee et al., 2005). Firing is lower during quiet waking and absent during sleep, when postural tone is low. In rapid eye movement [REM] sleep with low postural tone, these neurons are silent, despite the muscle twitches occurring during this state. Evidence from studies using FOS-immunoreactivity as a marker of neuronal activity indicates that hypocretin neurons exhibit 24 h patterns of activity, which vary according to behavioral state and in relation to food-related cues or reward-seeking behavior (Sakurai, 2003; Mieda et al., 2004; Paneda et al., 2005; Harris et al., 2005). Hypocretin neurons thus antagonize sleep and muscle atonia, thereby supporting arousal in the context of consummatory behaviors.

HYPOCRETIN NEURONS INTEGRATE MULTIPLE CIRCADIAN-PHOTIC, NUTRIONAL-METABOLIC, AND NEUROENDOCRINE INFLUENCES

Hypocretin neurons in hypothalamic slices *in vitro* display a rather low membrane potential of about −50 mV (Li et al., 2002; Sakurai, 2003; Horvath and Gao, 2005). They are excited by numerous somatic glutamatergic inputs, ATP, acetylcholine, ghrelin, GLP-1, CRF, AVP, OXY, neurotensin, and CCK. Their intrinsic activity is inhibited through presynaptic metabotropic glutamate autoreceptors (mGluR) and negative feedback through GABAergic, catecholaminergic, and serotonergic neurons, as well as by glucose, leptin, NPY, and adenosine.

A major inhibitory GABA-galaninergic pathway to the posterior hypothalamus, including hypocretin and histamine but not melanin-concentrating hormone (MCH) neurons, emanates from sleep-active neurons in the ventrolateral preoptic area (VLPO), as shown in Figure 1 (Saper et al., 2001;

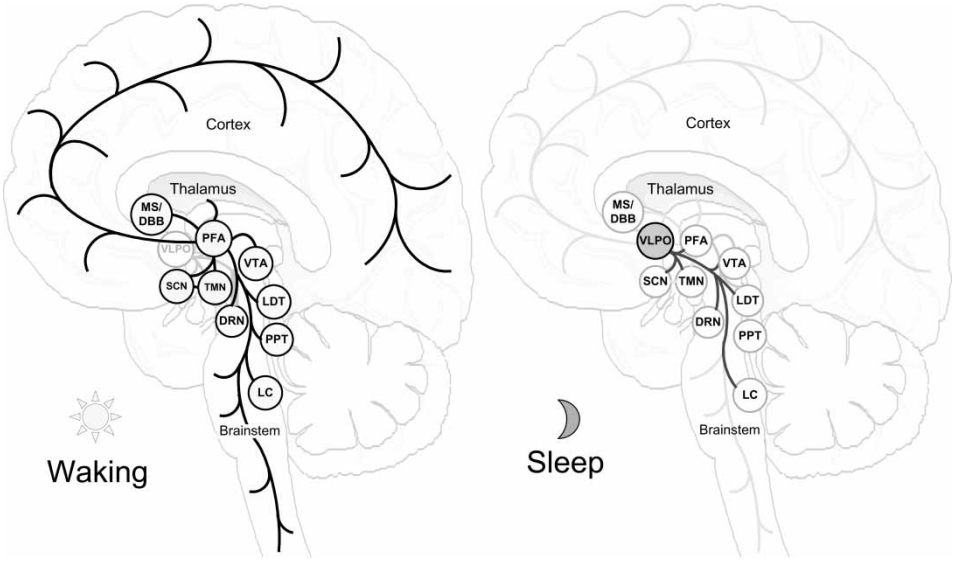

FIGURE 1 Key components of brain arousal and sleep control systems. MS/DBB = medial septum/diagonal band of Broca (acetylcholine); SCN = suprachiasmatic nucleus (glutamate, AVP); PFA = perifornical area (hypocretins); VLPO = ventrolateral preoptic area (GABA, galanin); TMN = tuberomamillary nucleus (histamine); VTA = ventral tegmental area (dopamine); LDT/PPT = laterodorsal/pedunculopontine tegmentum (acetylcholine); DRN = dorsal raphe nuclei (serotonin); LC = locus coeruleus (noradrenaline).

Eriksson et al., 2004; Sergeeva et al., 2005). The ascending arousal system, in turn, is inhibited by the VLPO. These neural circuits form a bistable flip-flop switch (analogous to an electronic circuit) that gates rapid transitions between behavioral states (Saper et al., 2001). Activity of hypocretin neurons in this switch is required for the maintenance of behavioral state bistability (Mochizuki et al., 2004). In other words, these neurons set the threshold for state transitions preventing intermediate states and inappropriate transitions between states, such as those occurring in narcolepsy.

Adenosine has been proposed as a homeostatic sleep pressure factor that accumulates in the brain during wakefulness and motor activity (Huston et al., 1996). Hypocretin neurons express adenosine receptors and, as evidenced by FOS-immunoreactivity, are sensitive to the wake-promoting actions of stimulant doses of the adenosine receptor antagonist caffeine (Murphy et al., 2003), the most frequently used wake-promoting drug worldwide. This suggests that hypocretin neurons may relay homeostatic influences to the behavioral state control systems and to the sleep-wake switch.

Timing of behavioral state transitions is influenced by endogenous and environmental factors, such as food and stress (Saper et al., 2001; Mieda et al., 2004; Paneda et al., 2005). The suprachiasmatic nucleus (SCN) confers a circadian timing signal to the brain and other body tissues by

humoral factors and neuronal connections (Buijs and Kalsbeek, 2001; Hastings et al., 2003). Lesion studies indicate that circadian rhythmicity of most of the behavioral state control systems (except dopamine) persists under constant darkness, suggesting that they are all entrained in rhythmicity derived from the SCN.

Both hypocretin and histamine neurons in the lateral hypothalamus receive direct glutamatergic (and probably humoral) input from the SCN (Abrahamson et al., 2001; Deurveilher and Semba, 2005). In contrast, the SCN exhibits no direct connections with other wake- or sleep-active neurons. The medial preoptic area, subparaventricular zone, and dorsomedial hypothalamus relay largely indirect connections (Saper et al., 2001; Deurveilher and Semba, 2005). Thus, the hypocretin and histamine systems seem to play specific roles in the expression of circadian rhythms. Hypocretins exert dose-dependent effects on the discharge rate of SCN neurons *in vitro* (Farkas et al., 2002) and mediate signals to the pineal gland (Mikkelsen et al., 2001), which contains high densities of hypocretin fibers and receptors (Fabris et al., 2004). The levels of hypocretin-1 in the brain vary according to behavioral state, and lesions of the SCN eliminate the daily rhythm of hypocretin-1 release (Mignot et al., 2002; Siegel, 2004). Notably, mice lacking mPER also lack 24 h variation of hypocretin level and its accumulation during the active period (Turek et al., 2005). Day-night rhythms of ghrelin levels are blunted in these animals, resulting in obesity and metabolic syndrome.

Dampened 24 h variations of hypocretin levels have been reported in depressed human subjects (Mignot et al., 2002; Siegel, 2004); whereas, addiction is associated with a dysfunction of mPER2, glutamatergic neurotransmission (Spanagel et al., 2005), and/or a hyperfunction of the hypocretin system (Harris et al., 2005). Animals lacking endogenous histamine (HDC-KO) exhibit disrupted *mPer1* and *mPer2* mRNA rhythms in the cortex and striatum, but not in the SCN, suggesting that both histamine and hypocretin are involved in the output pathway or feedback route from behavior to the circadian pacemaker in the SCN (Abe et al., 2004).

HYPOCRETIN ACTIONS

Hypocretins act on G_q-protein-coupled receptors signaling through phospholipase C (PLC)- and calcium-dependent as well as calcium-independent transduction pathways (Kukkonen et al., 2002; Selbach et al., 2003). These include activation of electrogenic sodium-calcium exchangers (NCX) and a non-specific cationic conductance, likely channels of the transient receptor potential canonical-(TRPC) type activation of L-type voltage-dependent calcium channels, closure of G-protein-activated inward rectifier potassium channels (GIRK), and activation of protein kinases, including protein kinase C (PKC), protein kinase A (PKA), and

mitogen-associated protein kinase (MAPK). Postsynaptic actions of hypocretins on their numerous neuronal targets throughout the CNS are almost entirely excitatory (De Lecea et al., 1998).

HYPOCRETINS ORCHESTRATE BEHAVIORAL STATE CONTROL SYSTEMS

The densest innervation with hypocretin-containing nerve fibers and prominent excitatory postsynaptic actions of hypocretins are found in noradrenergic neurons in the locus coeruleus (Hagan et al., 1999) and cholinergic neurons in the forebrain and lateral dorsal tegmentum (LDT) (Burlet et al., 2002) (Figure 1). We studied the action of histamine-, serotonin-, and dopamine-containing neurons in some detail. Histamine neurons in the tuberomamillary nucleus (TMN) of the posterior hypothalamus display a pacemaker-like activity during waking and are inhibited during sleep (Haas and Panula, 2003). These neurons are not only the closest neighbors of hypocretin neurons but are also close functional partners (Huang et al., 2001; Saper et al., 2001; Eriksson et al., 2001; Parmentier et al., 2002; Eriksson et al., 2004; Sergeeva et al., 2005). Excitation of TMN neurons near body temperature in slices occurs mainly through activation of electrogenic Na^{+}/Ca^{2+}-exchangers, a mechanism of action that is rarely described but may be much more frequent than commonly thought, given that it escapes the usual methods of investigation. In addition, hypocretin-1 modulates GABAergic inputs from the VLPO to TMN neurons, a proposed locus of action of commonly used anesthetics (Eriksson et al., 2004). This action is gated by dynorphin, which is highly co-expressed in hypocretin neurons together with neuronal activity-regulated pentraxin (Narp), a pentraxin involved in aminohydroxy-methylisoxazol-propionic acid (AMPA) receptor clustering. Activation of a non-specific cationic conductance, probably TRPC-type channels (Sergeeva et al., 2005), by Hcrt1 receptors depolarizes and activates serotonergic neurons in the raphe nucleus, convergent with activation of histamine H1R and noradrenaline alpha1R receptors (Brown et al., 2002). Dopaminergic and GABAergic neurons in the substantia nigra and the ventral tegmental area display a more complex pattern of hypocretin receptor expression and mechanisms of action (Korotkova et al., 2003). Both GABAergic and dopaminergic neurons in the substantia nigra (SN) and ventral tegmental area (VTA) are strongly excited by hypocretins, some exhibiting burst firing, which *in vivo* encodes prediction of rewards (Harris et al., 2005). Moreover, unlike the other above mentioned transmitter systems, dopamine levels in brain areas innervated by the VTA do not show 24 h variation, suggesting that the actions of hypocretins in the VTA may serve purposes other than the control of sleep and wakefulness (Harris et al., 2005). Hypocretins act on numerous targets throughout the CNS and beyond; for instance, they

directly activate nonspecific thalamic and layer VIb cortical neurons (Bayer et al., 2004). Finally, hypocretins promote synaptic plasticity in cortical (Selbach et al., 2004) and subcortical targets (Horvath and Gao, 2005). The mechanistic and molecular signature of a remarkable protein synthesis-dependent long-term potentiation (LTP) of synaptic transmission in the mouse hippocampus points to roles of hypocretins in neurodegeneration and memory functions (Harris et al., 2005). Moreover, expression of functional hypocretin receptors in human CD34+ hematopoietic stem cells (Steidl et al., 2004) suggests an even more general role in tissue homeostasis, plasticity, and immunity.

NARCOLEPSY—LOSS OF TIMING AND MAINTENANCE OF SLEEP AND WAKING

Lesions of sleep-active GABAergic neurons in the VLPO of the anterior hypothalamus cause a fatal insomnia. Destruction of the posterior hypothalamus, including the histaminergic and hypocretinergic nuclei, produces a syndrome of hypersomnia and cachexia, encephalitis lethargica (Von Economo, 1930).

Hypofunction of the hypocretin system, mostly as a consequence of an autoimmune attack against hypocretin neurons, causes narcolepsy, a debilitating inability to separate and consolidate sleep and waking (Mignot et al., 2002; Siegel, 2004). Daytime sleepiness and sudden loss of muscle tone during waking (cataplexy), normally experienced only during REM sleep, match with disrupted nighttime sleep and hypnagogic hallucinations during sleep paralysis. Potent triggers of cataplexy are emotionally arousing primary rewards, such as food presentations in animals or laughter and joy in humans. Hypocretin neurons seem to be indispensable for the maintenance of behavioral state bistability (Saper et al., 2001) as well as motor activity associated with appetitive behaviors (Siegel, 2004; Lee et al., 2005; Harris et al., 2005). Histamine neurons in Hcrt2 receptor-deficient narcoleptic dogs, in contrast to other monoaminergic "REM-off" cell groups, remain active during cataplexy, suggesting their control of quiet waking and novelty (Huang et al., 2001; Parmentier et al., 2002); whereas, noradrenergic and serotonergic neurons control muscle tone in waking and its loss in REM sleep and cataplexy (Siegel, 2004).

CONCLUSION

The hypocretin system integrates circadian-photic and nutritional-metabolic influences to regulate the timing and consolidation of behavioral, arousal, motivational, and nutritional states. This impacts brain functions and whole-body homeostasis in health and disorders of sleep, energy metabolism, movement, mood, and memory.

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