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Interaction between Orexin Neurons and Monoaminergic Systems

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Abstract

Orexins have received a lot of attention as potent endogenous arousal-promoting peptides, and orexin receptor antagonists have shown clinical efficacy for the treatment of insomnia. Orexin neurons are thought to act primarily on monoaminergic neurons to maintain arousal and vigilance. In this chapter, we discuss the functional interaction between monoaminergic systems, including noradrenaline, serotonin and histamine, and orexin neurons, as well as interactions between the acetylcholine system and the orexin neurons, focusing, in particular, on their function in the regulation of sleep-wakefulness states. Orexin also has close interactions with the dopaminergic system, and many studies have suggested roles of orexin signaling in the reward system and roles for orexins in drug addiction.

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Introduction

Orexins are hypothalamic neuropeptides that play a highly important role in the maintenance of wakefulness. Orexin-A and orexin-B, which are derived from a common precursor peptide, bind to two subtypes of G-protein-coupled receptors, the orexin 1 receptor (OX₁R) and the orexin 2 receptor (OX₂R). Orexins potently increase wakefulness and suppress both non-rapid eye movement and rapid eye movement (REM) sleep [1].

In various mammalian species, including mice, rats, dogs and humans, loss of orexin neurons results in narcolepsy, a condition characterized by excessive daytime sleepiness, abnormally brief transitions to REM sleep from wakefulness (sleep-onset REM periods), and cataplexy, characterized by sudden loss of bilateral skeletal muscle tone [2–4]. This

highlights a critical role of orexins in the maintenance of wakefulness. Animal models first suggested the involvement of orexin dysfunction in narcolepsy. Mice with a targeted deletion of the *prepro-orexin* gene (*orexin*^{-/-} mice) and mice with genetic ablation of orexin neurons both showed a narcoleptic phenotype, characterized by abrupt behavioral arrests with muscle atonia (cataplexy), fragmented sleep-wakefulness states, and direct transitions from wakefulness to REM sleep [5, 6]. In addition, functionally null mutations in the *OX₂R* gene were found in familial narcoleptic dogs [7]. Consistently, *OX₂R*^{-/-} mice are also narcoleptic, although their phenotype is significantly milder than that of *orexin*^{-/-} mice or *orexin/ataxin-3*-transgenic mice [8]. In contrast, *OX₁R*^{-/-} mice do not show any overt abnormalities in sleep-wakefulness states [1, 9]. These observations suggest that the *OX₂R*-mediated pathway has a pivotal role, although *OX₁R* has an additional role in the maintenance of wakefulness. Orexin receptors are abundantly expressed in histaminergic, noradrenergic, and serotonergic neurons, as well as cholinergic neurons [10], which play critical roles for sleep-wakefulness regulation.

The discovery of a causal link between loss of orexin signaling and narcolepsy has brought about the possibility of novel therapies for the disorder. Indeed, we have demonstrated that acute intracerebroventricular administration of orexin-A maintained wakefulness, suppressed sleep, and inhibited cataplectic attacks in *orexin/ataxin-3* transgenic mice [11]. In addition, a non-peptide *OX₂R* agonist was shown to have potential value for treating narcolepsy [12, 13]. Especially, TAK925 was reported to ameliorate symptoms of both type-1 and type-2 narcolepsy [14, 15]. Orexin receptor agonists might also be beneficial for treating conditions of excessive daytime sleepiness. Likewise, dual orexin receptor antagonists, which antagonize both orexin receptors with similar affinity, have shown clinical efficacy for the treatment of insomnia [16, 17]. Understanding the function of orexin receptors, in particular their roles in regulating monoaminergic neurons, is important for elucidating the mechanism by which the orexin system maintains wakefulness, as well as for understanding the effect of orexin receptor agonists/antagonists for treating hypersomnia and insomnia, respectively. Here, we discuss the functions of orexin and orexin receptors in the regulation of monoaminergic neurons, including noradrenergic, serotonergic, dopaminergic, and histaminergic neurons, as well as cholinergic neurons, all of which are localized in the brain stem nuclei. The role of these monoaminergic/cholinergic neurons in sleep-wakefulness regulation has been clarified by the correlation of their spontaneous firing frequency with the sleep-waking cycle [18–20]. Monoaminergic release in several cortical and subcortical sites was also correlated with the sleep-waking cycle [21–23].

An Anatomical View of the Interactions between Orexin Neurons and Monoaminergic Systems

Orexin neurons are distributed within the lateral hypothalamus and send projections throughout the brain and spinal cord. In particular, there are dense innervations to nuclei containing monoaminergic and cholinergic neurons in the brainstem and the hypothala-

Fig. 1. Schematic drawing of input and output systems of orexin neurons. Orexin neurons receive abundant input from the regions implicated in stress and emotional processing. Neurons in the preoptic area, including those in the ventrolateral preoptic nucleus (VLPO) send especially rich innervations to orexin neurons. Orexin neurons send widespread projections throughout the brain, with especially dense projections to the noradrenergic, serotonergic, and histaminergic neurons in the posterior hypothalamus/brain stem regions. Arc, arcuate nucleus; DMH, dorsomedial hypothalamus; DR, dorsal nucleus raphe; GABA, gamma-aminobutyric acid; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; LHA, lateral hypothalamic area; NPY, neuropeptide Y; PPN, pedunculopontine tegmental nucleus; REM, rapid eye movement; SCN, suprachiasmatic nuclei; SNr, substantia nigra reticulata; TMN, tuberomammillary nucleus; VTA, ventral tegmental area.

mus, including noradrenergic neurons of the locus coeruleus (LC), serotonergic neurons of the dorsal and median raphe nuclei (DR and MnR), histaminergic neurons of the tuberomammillary nucleus (TMN), and cholinergic neurons of the laterodorsal tegmental nucleus (LDT) and pedunculopontine tegmental nucleus (PPT) [1].

In situ hybridization studies have suggested that two subtypes of orexin receptors are differentially expressed in monoaminergic nuclei. Our previous work showed that within the LC, all *vesicular monoamine transporter 2 (VMAT2)*-positive noradrenergic neurons exhibited intense *OX₁R* expression, whereas *OX₂R* mRNA was exclusively detected

in VMAT2-negative, non-noradrenergic neurons within the same nucleus [10]. In the TMN, on the contrary, all VMAT2-positive histaminergic neurons expressed *OX₂R* mRNA but not *OX₁R*. In the DR and MnR, both *OX₁R* and *OX₂R* mRNAs were expressed in approximately 90% of VMAT2-positive serotonergic neurons, suggesting that the majority of serotonergic neurons in these areas express both *OX₁R* and *OX₂R*; whether or not these receptors are expressed in the same neurons has not as yet been clearly shown. *Gad1*-positive cells in the DR and MnR also expressed *OX₁R* or *OX₂R* mRNA, suggesting that GABAergic interneurons in the raphe nuclei express both orexin receptors. In the LDT and PPT, all vesicular acetylcholine transporter (*VACht*)-positive cholinergic neurons expressed *OX₁R* but not *OX₂R* mRNA, suggesting that cholinergic neurons in these regions exclusively express *OX₁R*. Both *OX₁R* and *OX₂R* mRNA were also expressed in GABAergic and neurochemically unidentified neurons in the LDT and PPT. These anatomical observations using in situ hybridization histochemistry suggest close interaction between orexin neurons and monoaminergic/cholinergic systems, and that orexin neurons stabilize wakefulness by regulating these monoaminergic and cholinergic neurons (Fig. 1).

Function of *OX₁R* in the Locus Coeruleus

Electrophysiological studies have shown that orexin-A and orexin-B increase the firing rates of monoaminergic neurons in the LC [24]. Orexin neurons send abundant projections to the LC. Orexin neurons that send projections to the LC receive input from a wide variety of brain regions, including the nucleus accumbens and the amygdala, suggesting the role of this connection in emotive function (Fig. 2). Application of orexin-A directly into the LC has been reported to suppress REM sleep and increase wakefulness [25]. *OX₁R*^{-/-} mice showed mild fragmentation of sleep-wakefulness states, which was significantly less than the wakefulness fragmentation observed in *OX₂R*^{-/-} mice. The highest fragmentation was observed in *orexin*^{-/-} mice and *OX₁R*^{-/-};*OX₂R*^{-/-} mice [1, 26]. This suggests that *OX₁R* is indispensable for the maintenance of wakefulness in the absence of *OX₂R* [10].

We observed that the maintenance of wakefulness in *OX₁R*^{-/-};*OX₂R*^{-/-} mice was significantly improved by noradrenergic neuron-selective restoration of *OX₁R* in the LC to a level of wakefulness comparable to that in *OX₂R*^{-/-} mice [27]. However, it was still significantly impaired as compared to that in wild-type mice, suggesting that LC noradrenergic neurons play a significant, but not the only, role for maintenance of wakefulness by orexin. Other regions are also required for the normal regulation of wakefulness. Consistently, a causal relationship between the firing of noradrenergic neurons in the LC and transitions from sleep to wakefulness has been shown by an optogenetic approach [28].

We found that a subpopulation of noradrenergic neurons in the LC projecting to the lateral amygdala (LA) receive synaptic input from orexin neurons [29]. Pharmacogenetic/

Fig. 2. Depiction of input of orexin neurons that send innervations to the locus coeruleus by the cTRIO approach [63]. We injected *CAV2-DIO-Flp* in the locus coeruleus, and then *AAV2-DIO-TVA-mCherry* and *AAV2-DIO-RG* in the LHA in orexin-Cre mice. After 14 days, *SAD-dG-GFP(EnvA)* was injected into the LHA to depict input to orexin neurons that send innervations to the LC. We observed similar distributions of input neurons with retrograde tracing of orexin neurons [45]. Acb, nucleus accumbens; BST, the bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; cTRIO, cell type-specific tracing; DR, dorsal raphe; LHA, lateral hypothalamic area; LPO, lateral preoptic nucleus; PVH, paraventricular hypothalamic nucleus; SON, supraoptic nucleus.

optogenetic silencing of this circuit, as well as acute blockade of OX_1R , decreased conditioned fear responses. In contrast, optogenetic stimulation of this circuit potentiated freezing behavior against a similar, but distinct, context or cue. An increase in orexinergic tone by fasting also potentiated the freezing behavior and LA activity, which were blocked by pharmacological blockade of OX_1R in the LC. These observations suggest that the circuit involving orexin, LC and LA neurons mediates fear-related behavior, and that excitation of this pathway may cause fear generalization [29].

Function of OX₁R and OX₂R in the Raphe Nuclei

Orexin-A and orexin-B increase the firing rates of serotonergic neurons in the DR [30, 31]. In addition to the direct excitatory effects, orexins act indirectly to activate local inhibitory GABAergic input to serotonergic neurons [31]. This is consistent with expression of orexin receptors in GABAergic neurons of the DR/MnR [10].

Cataplexy frequently occurs in *OX₁R*^{-/-}/*OX₂R*^{-/-} mice but rarely in *OX₂R*^{-/-} mice, and never in *OX₁R*^{-/-} mice [8, 9, 26], suggesting that both OX₁R and OX₂R-mediated pathways play roles in the inhibition of cataplexy. Consistently, DR serotonergic neurons, which express both OX₁R and OX₂R, play a critical role in the prevention of cataplexy-like episodes [10].

The fact that DR serotonergic neurons show greatly reduced activity during cataplexy in narcoleptic dogs, and that selective serotonin reuptake inhibitors are effective for cataplexy, further support the important role of orexin signaling in DR serotonergic neurons in the inhibition of cataplexy [32]. Serotonergic neurons in the DR send dense projections to the PPT/LDT, substantia nigra pars compacta, and the amygdala – regions implicated in the regulation of REM sleep [1]. In particular, cataplexy is often triggered by strong emotions, and thus serotonergic modulation of the amygdala (a region important for emotion processing) may be relevant to the suppression of cataplexy. Consistently, the amygdala contains cataplexy-related neurons [33], as well as REM-active neurons that are inhibited by DR stimulation [34].

We found that restoration of OX₂R expression in the DR of *OX₁R*^{-/-}/*OX₂R*^{-/-} mice significantly decreased cataplexy-like attacks [35]. In addition, optogenetic excitation of serotonergic neurons in the DR or serotonergic fibers in the basolateral amygdala reduced cataplexy-like attacks, suggesting that orexin-mediated excitation of serotonergic neurons in the DR inhibits cataplexy-like attacks by acting on the basolateral amygdala [35]. Stimulating serotonin nerve terminals reduced amygdala activity. In addition, chemogenetic inhibition of the amygdala drastically decreased cataplexy-like episodes, whereas chemogenetic activation increased them [35]. These results suggest that DR serotonin neurons, as a downstream target of orexin neurons, inhibit cataplexy by reducing the activity of amygdala.

Function of OX₂R in the Tuberomammillary Nucleus

Histaminergic neurons in the TMN are activated by orexins [36–38]. Mochizuki et al. [26] reported that focal restoration of OX₂R in neurons of the posterior hypothalamus, including the TMN, ameliorated the fragmentation of wakefulness in OX₂R-null OX₂R transcription-disrupted (TD) mice, in which a loxP-flanked transcription disrupter gene cassette prevents expression of functional OX₂R. However, no improvement of fragmentation of wakefulness in *OX₁R*^{-/-}/*OX₂R*^{-/-} mice was observed when OX₂R expression was restored in the TMN and adjacent parts of the posterior hypothalamus. This suggests that

OX₂R expression in the TMN may be sufficient to consolidate wakefulness only when OX₁R is expressed in the brain. Alternatively, OX₂R restoration in unidentified neurons in the posterior hypothalamus, other than the TMN, may play a role in the consolidation of wakefulness in OX₂R TD mice. This is consistent with a finding that orexin-mediated sleep-to-wake transitions do not depend on the TMN histaminergic neurons [39].

Interaction between Orexin and Cholinergic System

Cholinergic neurons in the brain stem, which are located in the LDT/PPT, are well-known to play important roles in the regulation of sleep-wakefulness states. In addition, as mentioned earlier, orexin neurons send abundant projections to these regions, and LDT/PPT express orexin receptors. However, it has remained unclear how orexin affects the activity of cholinergic neurons in the LDT/PPT. These neurons express OX₁R, suggesting that orexin has excitatory effects on these cells, which is supported by an *in vitro* electrophysiology study [40]. Orexin neurons have also been shown to reciprocally interact with the basal forebrain cholinergic neurons [41, 42], which play an important role in attentive functions. Orexin neurons have been thought to partly promote arousal and attention through their projections to the basal forebrain cholinergic neurons [43].

Feedback Regulation of Orexin Neurons by Monoaminergic Systems

Our previous studies showed that noradrenaline (NA) and serotonin (5-HT) directly inhibit orexin neurons [44, 45], constituting negative feedback regulatory mechanisms for orexin neurons, although physiological relevance of these connections has not yet been revealed. However, NA and 5-HT also inhibit GABAergic neurons that send direct inhibitory projections to orexin neurons [45], suggesting that monoamines influence the activity of orexin neurons in a complex manner.

Orexin and Dopaminergic System

Orexin neurons send dense projections to the ventral tegmental area (VTA) with its dopaminergic neurons that themselves send rich innervations to the nucleus accumbens [46]. This suggests an interaction of the orexin with the reward system. The VTA expresses both subtypes of orexin receptors, with dopaminergic neurons predominantly expressing OX₁Rs [47, 48]. Orexin signaling in the VTA has been implicated in reinforcement and reward-related processes via actions on VTA-dopamine neurons [49]. Orexin neurons may be activated by external reward-related stimuli and send information to the VTA to induce reinstatement of drug seeking via the OX₁R [50]. Orexin neurons are excited during the behavioral expression of preferences for cues associated with reward [51],

and administration of orexins, either intracerebroventricularly or locally into the VTA, can reinstate previously-extinguished drug-seeking or food-seeking behavior in rodents [51, 52].

There is much work to show that orexin neurons also play a part in addiction-related behaviors in rodents in response to different drugs of abuse [53–55]. Primarily, orexins are involved in the modulation of highly motivated reward-seeking, especially when this seeking is triggered by external cues. The OX₁R appears to mainly mediate this function. For instance, orexin-A has been shown to promote motivation via OX₁R signaling in a study in rats in which high levels of effort were required for seeking addictive drugs or highly palatable food in self-administration protocols [56].

In other experiments in rodents, the OX₁R antagonist SB-334867 attenuated the expression of amphetamine-induced conditioned place preference [57] as well as the acquisition of locomotor sensitization to cocaine [58] and the expression of locomotor sensitization to amphetamine [59]. Together, these results suggest that orexins are essential for reward-seeking and primarily support motivated behavior which requires a high level of arousal [60].

Conclusion

Orexin neurons strongly excite monoaminergic neurons in the brainstem regions. Orexin neurons receive abundant input from a wide variety of brain regions, including the limbic system, paraventricular nucleus and preoptic areas [45]. Orexins also play an important role in the regulation of the reward system through modulation of the dopaminergic system in the VTA [61]. Orexin neurons have also been shown to be regulated by humoral factors, including leptin, glucose, and ghrelin [62]. These observations suggest the orexin neurons integrate animals' external and internal states to maintain vigilance levels accordingly, at least in part, through the regulation of monoaminergic neurons. Further understanding of input and output architecture of orexin neurons that send innervations to particular monoaminergic systems would provide us with further information about the mechanisms by which orexin neurons regulate monoaminergic neurons.

Key Take-Home Points

- Orexin neurons strongly excite monoaminergic neurons in the brain stem regions.
- These connections play important roles in the regulation of sleep and wakefulness.

Conflict of Interest Statement

T.S. and M.Y. received consultancy fees from Idorsia Pharmaceuticals Ltd during the development of this book. The authors have no further conflicts of interest to declare.

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Author Contributions

All authors were involved in the development and review of the chapter, approved the final version to be published and take responsibility for all aspects of the work.

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