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Abstract

Lateral hypothalamic neuropeptides, orexins, have been recognized as one of the most important regulators of sleep/wakefulness states. Besides, these peptides are also regarded as an important factor that regulates feeding behavior, owing to their localization within the lateral hypothalamic area, the classic "feeding center", pharmacological activities, and the fact that prepro-orexin mRNA is upregulated when animals are fasted. This review summarizes the role of orexins in the regulation of feeding behavior and body weight homeostasis in relation to other systems that involve orexinergic neurotransmission.

Introduction

Orexins are hypothalamic neuropeptides identified in 1998[1]. Several studies showed that orexin deficiency causes narcolepsy in humans and animals, implicating these hypothalamic neuropeptides in play a critical role in the regulation of sleep/wakefulness states[2-6]. However, orexins were initially recognized as regulators of feeding behavior, firstly because of their exclusive production in the lateral hypothalamic area (LHA), a region known as the "feeding center", and secondly because of their pharmacological activity; intracerebroventricular (ICV) injection of orexins induced feeding behavior in rats and mice[1,7-9]. Recent studies suggested that further orexins play further roles in the coordination of emotion, energy homeostasis, reward system, drug addiction, and arousal[10-17]. This review focuses especially on the role of orexins in the regulation of

feeding, body weight and energy homeostasis in relation to other systems in which orexins are shown to be involved (Fig. 1).

1.Overview of the orexin system

1-1.Orexins and their structures

In 1998, we identified novel neuropeptides, orexin A and orexin B, from rat brain extracts as two endogenous ligands for two orphan G-protein-coupled receptors by a method so-called "reverse pharmacology", which utilized receptor-expressing cell lines as the assay system[1]. Molecular cloning studies showed that both orexin A and orexin B are derived from a common precursor peptide, *prepro-orexin*. An mRNA encoding the same precursor peptide was independently identified by de Lecea *et al.* as a hypothalamus-specific transcript[18]. de Lecea *et al.* predicted that the transcript encoded a polypeptide precursor that is proteolytically cleaved to produce two isopeptides, and named them as hypocretin-1 and hypocretin-2 (corresponding to orexin A and orexin B, respectively).

Orexin A and orexin B constitute a novel distinct peptide family, showing no significant homology with any other peptides[19]. Structural analysis of purified peptide showed that orexin A is a 33-amino-acid peptide with an N-terminal pyroglutamyl

residue, two intra-chain disulfide bonds, and C-terminal amidation. This structure is completely conserved among mammalian species (human, rat, mouse, cow, sheep, dog and pig). Orexin B is a 28-amino-acid, C-terminally amidated linear peptide. Amino acid sequences of various species of orexin B show that there are several inter-species differences, although highly conserved. The C-terminal half of orexin B is very similar to that of orexin A, whereas the N-terminal half is more variable.

1-2 Transcriptional regulation of orexin

Prepro-orexin mRNA is highly specifically expressed by a population of neurons which are located in and around the LHA[1,20]. The expression of orexin has been shown to be upregulated by fasting[1], suggesting that the transcriptional regulatory sytem of orexin gene should include the mechanisms that restrict the expression of orexin mRNA in a selective population of neurons in the LHA, and that increase its expression during fasting. However, very limited information has been available to elucidate these mechanisms so far.

The 3.2-kb 5'-flanking region of the human *prepro-orexin* gene is sufficient for the specific expression in orexin neurons[20,21], and thus has been used as a promoter to drive specific expression in orexin neurons. We found two phylogenetically conserved

regions located 287-bp (orexin regulatory element (OE) 1) and 2.5-kb (OE2) upstream of the transcription initiation site in the human *prepro-orexin* gene. In transgenic mice, both OE1 and OE2 are necessary for expressing the human *prepro-orexin* gene in the LHA and for repressing its expression in the medial regions of the hypothalamus. The 57-bp core region of OE1 is critical for the spatial gene regulatory function of prepro-orexin gene in vivo, which contains crucial cis-acting elements regulating *prepro-orexin* gene expression specifically in the LHA[20].

Recently, the forkhead box transcription factor Foxa2, a downstream target of insulin signaling, was shown to regulate the expression of orexin[22]. During fasting, Foxa2 binds to orexin promoter to stimulate the expression. In fed and in hyperinsulinemic obese mice, insulin signaling leads to nuclear exclusion of Foxa2 and reduces expression of orexin. Constitutive activation of Foxa2 in the brain results in increased neuronal orexin as well as MCH expressions and increased food consumption, energy expenditure and insulin sensitivity. Conditional activation of Foxa2 through the T156A mutation also resulted in improved glucose homeostasis, decreased body fat and increased lean body mass. These results suggest that Foxa2 acts as a metabolic sensor in orexin and MCH neurons to integrate metabolic signals, adaptive behavior and physiological responses.

1-3.Orexin Receptors

The actions of orexins are mediated by two G-protein coupled receptors (GPCRs), orexin 1 and orexin 2 receptors (OX1R and OX2R, also known as Hcrtr1 and Hcrtr2). OX1R has one-order-of-magnitude greater affinity for orexin A over orexin B. In contrast, OX2R binds both ligands with similar affinities[1]. OX1R couples to $G_{q/11}$ class of G-protein. Activation of this pathway results in activation of phospholipase C to trigger the phosphatidylinositol cascade and influx of extracellular Na^+ and Ca^{2+} concentrations, presumably through activation of transient receptor potential (TRP) channels leading to depolarization of neurons. OX2R is shown to couples to both $G_{q/11}$ or G_i-classes of G-proteins in a neuronal cell line[23], although the physiological relevance of Gi-mediated pathway downstream of OX2R has not been identified.

OX1R and OX2R exhibit a partially similar but partially distinct and basically complementary distribution, suggesting that these receptors have different physiological roles through different neuronal pathways [24].

1-4.Orexin-producing Neurons

Orexin neurons, which have been assumed to number around 3,000 in rat brains, or 70,000 in human brains[18,25], are localized exclusively in the hypothalamus, including the lateral hypothalamic area (LHA), perifornical area, and posterior hypothalamus (PH) [18,25,26]. Several factors were shown to be colocalized in orexin neurons, including dynorphine, neuronal activity regulated rentraxin (NARP), delta-like 1 homolog (DLK-1), and neurotensin[27]. Orexin neurons also express vesicular glutamate transporter 2 (vGluT2), suggesting these neurons are also glutamatergic. Glutamatergic neurotransmission by orexin neurons was electrophysiologically demonstrated in tuberomammillary nucleus (TMN) histaminergic neurons using optogenetic stimulation[28], although the physiological relevance of the capability of orexin neurons to elicit fast glutamatergic neurotransmission on target neurons has remained unknown.

A number of factors that influence firing rates or membrane potential of orexin neurons have been identified (Table 1). Several humoral factors that are implicated in energy homeostasis were found to affect the activity of orexin neurons. Cholecystokinin (CCK-8S) and a mixture of amino acids were shown to activate orexin neurons[29-32], whereas glucose, a BRS3 agonist and leptin inhibit them. These observations suggest that orexin neurons might be sensing peripheral metabolic states.

1-5.Physiological roles of orexins

Orexins have been recognized as multi-tasking peptides implicated in a variety of physiological functions, including the regulations of sleep/wakefulness states, feeding behavior, energy homeostasis, reward system, stress response, cognitive functions, emotional memory, endocrine function, thermogenesis and the autonomic nervous system. Among these, the most significant physiological role of orexins is thought to be that in the regulation of sleep/wakefulness states. This role was highlighted by the findings that orexin deficiency causes sleep disorder narcolepsy in humans and animals[2-5,12]. Please refer to other reviews on these functions[33-35], since this review would focus on the role of orexins in the energy homeostasis.

2. Roles of orexin in the regulation of feeding behavior

This section discusses the role of orexins in the regulation of feeding behavior, which is a main focus of this review.

2-1 Orexin and Feeding Behavior

The role in the regulation of feeding behavior was the first described physiological function of orexins[1]. We first reported the orexigneic effect of intracerebroventricular administration of orexin A and orexin B in rats. This effect was validated by several

subsequent studies in several species[33]. Furthermore, antibody for orexin or an antagonist of OX1R was shown to decrease food intake when administered centrally[36,37]. Consistently, orexin deficient mice show significantly decreased food intake[19,38].

One of the proposed functions of the orexin system in the regulation of energy homeostasis is to integrate metabolic state in the wakefulness to support feeding behavior[11,34,35]. Our group showed that mice lacking orexin neurons do not show an increase in wakefulness or locomotor activity in response to starvation[11]. This suggests that orexin plays an important role in evoking appropriate behavior in response to negative energy balance.

The first paper describing the identification of orexin had already reported that *prepro-orexin* mRNA was upregulated when animals were fasted, suggesting that orexin neurons sense animal's fasting status[1]. Subsequently, several reports suggested that orexin neurons are inhibited by glucose, triglycerides, and amino acids[11,32,39-41]. Furthermore, orexin neurons are inhibited by leptin, while excited by ghrelin[11]. These observations suggest that orexin neurons are sensing these factors to monitor animals' nutritional states, and integrating this information, and evoking necessary level of arousal.

Orexin neurons might also directly affect neuronal circuits within the

hypothalamus that are implicated in the regulation of feeding behavior. Orexins were shown to inhibit the VMH glucoreceptor neurons, while excite the arcuate nucleus (ARC) NPY neurons and the LHA MCH neurons[42-44]. Furthermore, local application of orexin in the PVH, DMH, and LHA was shown to increase food intake[45,46]. A recent study showed that the area postrema and NTS are involved in the orexin mediated feeding[47]. Application of orexin A into the nucleus of accumbens shell was reported to increase feeding[48]. These studies suggest that orexin evokes feeding behavior through multiple pathways.

Orexin might also play an important role in the hedonic aspect of feeding. Orexin was shown to evoke motivation to feeding, especially for palatable food[49-51]. Consistently, OX1R is shown to be involved in the reward regulation by orexin[52-54]. Furthermore, the mu-opioid receptor agonist, DAMGO (D-Ala(2)-N-MePhe(4)-Gly-ol(5)-enkephalin) -induced feeding in the NAc shell was shown to be dependent on an activation of OX1R[55]. These observations suggest that orexins are involved in the regulation of feeding through multiple pathways.

2-2. Roles of orexin in the regulation of body weight homeostasis

While orexins play a role in regulating feeding behavior, they are also involved in the

regulation of energy expenditure, which affects body weight homeostasis.

Narcolepsy patients were shown to have increased incidence of obesity despite being hypophagic, which is consistent with animal studies that showed orexin deficiency results in obesity in mice[56]. Obesity with BMI value over 30 is twice more common in narcolepsy patients than in general population[56]. Furthermore, narcoleptic patients were shown to be more obese than patients with idiopathic hypersomnia, suggesting that sleepiness and reduced activity in narcoleptic patients are not sole cause for the obesity[56].

Orexins have unique characteristics in feeding and energy expenditure: They increase feeding and energy expense simultaneously in response to various inner and outer environmental cues. Acute administration of orexin promotes feeding, although orexin deficiency in humans and mice is rather associated with obesity[6,19,38,57]. Conversely, transgenic mice with orexin overexpression show resistance to high-fat diet-induced obesity and insulin insensitivity[58]. Genetic study indicated that OX2R-mediated signaling predominantly mediates this effect[58]. Likewise, chronic central administration of an OX2R-agonist peptide inhibits diet-induced obesity[58]. Furthermore, orexin overexpression enhances the anorectic-catabolic effects of central leptin administration, while obese leptin-deficient mice are completely resistant to the

metabolic effects of orexin overexpression or OX2R agonist administration[58]. These observations suggest that enhanced orexin-OX2R signaling confers resistance to diet-induced features of the obesity and metabolic syndrome.

Orexins also increase insulin sensitivity through OX1R in VMH neurons, and enhance feeding-associated glucose utilization in skeletal muscle by increasing the activity of the sympathetic nervous system[59]. These observations suggest that orexins are monitoring animals' nutritional states and appropriately control arousal and peripheral metabolism through the regulation of the autonomic nervous system. Orexins were also shown to be involved in the diet-induced thermogenic function, which is important to resist weight gain when animals are exposed to increased caloric load[60].

Together, these observations suggest that in a broad sense, orexin neurons are involved in sensing the body's external and internal environments, and regulate feeding behavior, vigilance states, and metabolic functions accordingly, which is beneficial for survival.

Conclusion

Orexins were initially shown to be involved in the regulations of feeding behavior. Subsequently, their roles in the regulation of sleep/wakefulness states was highlighted by the discovery that orexin-deficiency causes narcoleptic phenotypes in animals and humans. Sleep and wakefulness are regulated to occur at appropriate times that are in

accordance with animals' internal and external environments. Avoiding danger and finding food, which are life-essential activities that are regulated by emotion, reward and energy balance, require vigilance and wakefulness. Orexin neurons receive abundant input from the limbic system[14,15], which might be important for increasing arousal in the emotionally-relevant situations (Fig. 1). Orexin neurons are also regulated by peripheral metabolic cues, including ghrelin, leptin and glucose, indicating that orexin neurons might provide a link between energy homeostasis and vigilance states. Together, these observations suggest that orexin neurons are involved in sensing the body's external and internal environments and regulate states of sleep and wakefulness accordingly, which is beneficial for survival.

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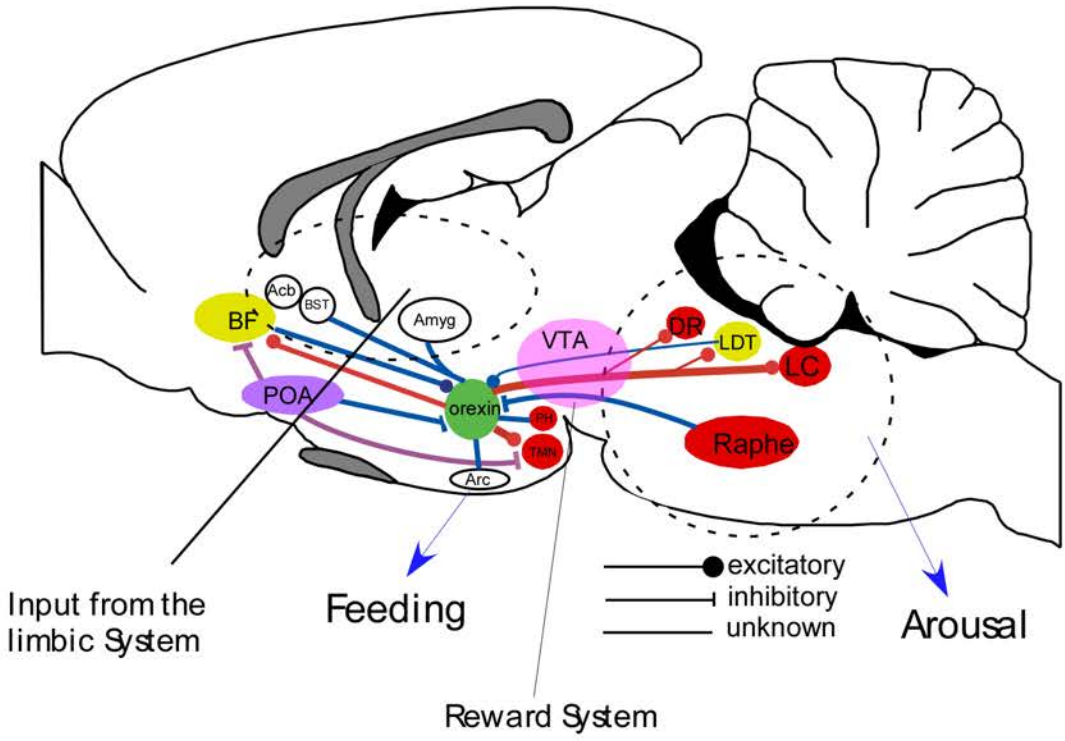
Fig. 1

Input and output of orexin neurons. Orexin neurons receive input from the limbic system, including the amygdala and bed nucleus of stria terminalis. These neurons also receive inhibitory projections from the preoptic area, a region which is thought to play an important role in sleep regulation. Orexin neurons are also sensing peripheral metabolic signals to monitor animal's energy balance. Orexin neurons send excitatory projections to the arousal center in the brain stem.

BF, basal forebrain; Acb, Nucleus accumbens; BST, bed nucleus of the stria terminalis; Amyg, amygdala; POA, preoptic area; Arc, arcuate nucleus; VTA, ventral tegmental area; DR, dorsal raphe; LDT, laterodorsal tegmental nucleus; LC, locus coeruleus; Raphe, raphe nuclei; TMN, tuberomammillary nucleus


Acb
BST
BF
Amyg
VTA
DR
LDT
LC
POA
orexin
PH
TMN
Raphe
Arc
Input from the
limbic System
Feeding
excitatory
inhibitory
unknown
Arousal
Reward System