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Review

Orexin receptors: Multi-functional therapeutic targets for sleeping disorders, eating disorders, drug addiction, cancers and other physiological disorders

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ABSTRACT

The orexin peptides (orexin A, orexin B) and their receptors (orexin receptor type 1, orexin receptor type 2) are involved in multiple physiological processes such as the regulation of sleep/wakefulness state, energy homeostasis and reward seeking. A result of this has been the development of small-molecule orexin receptor antagonists as novel therapies for the treatment of insomnia and drug addiction. Increased levels of signaling via the orexin peptide/receptor system may protect against obesity, while somewhat unexpectedly, orexins acting at orexin receptors induce dramatic apoptosis resulting in the significant reduction of cell growth in various cancer cell lines. Meanwhile, the orexin peptide/receptor system is also involved in cardiovascular modulation, neuroendocrine and reproduction regulation. This review summarizes the latest developments in deciphering the biology of orexin signaling as well as efforts to manipulate orexin signaling pharmacologically.

Contents

Abbreviations: AC, adenylyl cyclase; BAT, brown adipose tissue; BMP, bone morphogenetic protein; BST, bed nucleus of the stria terminalis; CB1R, cannabinoid receptor type 1; CNS, central nervous system; CREB, cAMP-response element binding protein; DRN, dorsal raphe nucleus; ERK, extracellular signal regulated kinase; FAA, food anticipatory activity; GABA, gamma-aminobutyric acid; GH, growth hormone; GPCR, G protein coupled receptors; HA, histamine; HIF1, hypoxia-inducible factor-1; 5-HT, 5-hydroxytryptamine; HPA, hypothalamic–pituitary–adrenal; HPG, hypothalamic–pituitary–gonadal; ICV, intracerebroventricular; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; LH, lateral hypothalamus; MAPK, mitogen-activated protein kinase; NAc, nucleus accumbens; RN, raphe nuclei; NSCC, nonselective cationic conductance; NPY, neuropeptide Y; OxA, orexin A; OxB, orexin B; OX1R, orexin receptor type 1; OX2R, orexin receptor type 2; pCREB, phosphorylated cAMP response element-binding protein; PH, posterior hypothalamus; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; REM, rapid eye movement; rRPa, rostral raphe pallidus; RVM, rostral ventromedial medulla; Smad, drosophila mothers against decapentaplegic protein; SN, substantia nigra; TGF-β, transforming growth factor-β; TMN, tuberomammillary nucleus; VTA, ventral tegmental area.

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1. Introduction

Orexin A (OxA) and orexin B (OxB) are neuropeptides, originally thought to promote feeding, which are produced in neurons of the lateral hypothalamus (also known as the feeding center of the brain [1]). This has conferred upon the orexins the alternative names of hypocretin 1 and hypocretin 2 [2,3]. Orexin peptides trigger many facets of physiology via their receptors, orexin receptor type 1 (OX1R) and orexin receptor type 2 (OX2R) (also known as Hctr1 and Hctr2 in accordance with hypocretin receptor type 1 and hypocretin receptor type 2), and these take the form of G protein-coupled receptors with seven transmembrane domains [2]. Over the fifteen years since it was first identified in 1998, the orexin system has been found to be involved in many physiological processes. Loss of the orexin-producing neurons leads to narcolepsy [4], a common sleeping disorder. This has inspired development of orexin antagonists as an approach to promote sleep and treat insomnia. Genetic ablation of orexin neurons results in obesity in mice [4], who then develop age-related glucose tolerance and insulin resistance [5]. In addition, orexin signaling strongly opposes diet induced obesity and insulin resistance through improving leptin sensitivity in rodents [6]. These observations have therapeutic implications for orexin agonists in the treatment of energy homeostatic disorders such as diet induced obesity and diabetes [7]. Orexin neurons also project into and innervate the drug and food reward associated brain regions such as the ventral tegmental area (VTA) and nucleus accumbens (NAc). Drug-seeking behavior was blocked by administration of an orexin antagonist [8], revealing that the use of orexin antagonists maybe a possible strategy for treating addiction. Orexin mediated activation of hypoxia-inducible factor-1 (HIF1) results in increased glucose uptake and higher oxidative energy metabolism as well as cell proliferation [7,9]. The literature has also shown that orexins induce dramatic apoptosis in many cell lines [10,11]. These findings suggest that the orexin receptors or their downstream effectors could be useful therapeutic options for the treatment of cancer.

2. Orexins, orexin neurons and the orexin receptors

OxA and OxB share 46% amino acid identity and are enzymatic cleavage products of a single 130 residue precursor, prepro-orexin. OxA is a 33 residue peptide (residues 28–66 of the prepro-orexin) with two intramolecular disulphide bridges within the N terminal, while OxB is a linear 28 residue peptide (prepro-orexin residues 69–97) [2,3,12]. The human orexin peptides are produced by less than 80,000 neurons exclusively located in the hypothalamus around the fornix, particularly, the lateral hypothalamus (LH). These neurons have anatomical projections into different brain regions [13–16], including the raphe nuclei (RN), the basal forebrain, the bed nucleus of the stria terminalis (BST), the olfactory area, the paraventricular nuclei, the substantia nigra (SN), the medullary reticular formation, the amygdaloid nuclei as well as the adenohypophysis and neurohypophysis of the pituitary [15–17]. Some of the densest projections are to the noradrenergic neurons in the locus coeruleus (LC), the serotonergic neurons in the dorsal raphe nuclei (DRN) of brain stem, the histaminergic neurons in the tuberomammillary nucleus (TMN) and the dopaminergic neurons in the VTA [15,18–20]. The extensive projections of the orexin neurons indicate that orexin system may have a role in physiological functions such as wakefulness, feeding and addiction, as well as regulation of cardiovascular and neuroendocrine system. In spite of all these mentioned above, the heaviest projections are still in accordance with regions in control of arousal (wakefulness).

The binding affinity of orexin peptides to the receptors has been determined using a competitive binding assay. OxA binds both OX1R and OX2R with high affinity (IC_{50} values are 20 nM and 38 nM, respectively), while OxB displays more selectivity (IC_{50} 420 nM with OX1R versus IC_{50} 36 nM with OX2R). In other words, OX1R is more amenable Q2 to selection, particularly when measuring calcium responses in CHO cells transfected with OX1R (EC_{50} of 30 nM for OxA as opposed to 2500 nM for OxB), despite having 64% amino acid homology with OX2R. In addition, neither OX1R nor OX2R has any significant affinity for other neuropeptides (neuropeptide Y (NPY), secretin and melanocortin) although they have some structural similarities to other receptors (NPY receptors and melanocortin receptors) [2,21]. The transmembrane domains 1, 3, and 5 and the amino terminus of the receptors account for the interaction with the orexin peptides. The transmembrane domain 3 is critical for receptor interactions with small molecule antagonists. This domain is also an important part of the small molecule binding pocket that is common to rhodopsin and β2-adrenergic receptors [22]. Consistent with the complex binding sites of orexin peptides upon orexin receptors, it is noticeable that both OX1R and OX2R exhibit slow kinetics in their response to orexin binding [23] (Table 1). Q3

As an important group of G protein coupled receptor (GPCR), OXRs are widely expressed throughout the central nervous system (CNS) and their distribution is consistent with the locations of orexin nerve terminals. The two groups of receptors show distinct distributions, but with a certain degree of overlap [24,25]. OX1R expression is in the cortical regions and brainstem nuclei, mainly involved in sleep and wake regulation as well as nuclei involved in reward signaling [2]. Some nuclei such as the LC only express OX1R mRNA. OX1R couples with Gq and induces intracellular calcium elevation mediated by phospholipase C (PLC) and also couples with Gs and Gi to mediate cAMP levels and non-selective cation channels [26–29]. OX1R signaling has been implicated in feeding, water intake, spatial learning and reward pathways [20,30–33]. OX2R is expressed only or mainly in histaminergic neurons of TMN, serotonergic neurons in the brainstem, the nucleus accumbens (NAc), the septal nuclei and the striatal nuclei, which mainly promote arousal [25,34–37]. OX2R is activated by both orexins A and B, and much like the OX1R, it couples with Gq to stimulate intracellular calcium via PLC; it is also able to activate Gs, Gi and ion channels [20,26,28,38,39].

With respect to orexin, much research has been concentrated upon the CNS; however orexins and OXRs are also expressed peripherally, although at relatively low levels. Moderate amounts of prepro-orexin and OX1R mRNA are found in the adrenal glands, testes and jejunum, high levels of orexins and OX2R mRNA in the adrenal cortex, and both receptor mRNAs in the adipose tissue, myenteric plexus of the small intestine, pancreas as well as in the retina (Fig. 1) [2,38,40–43]. The endocrine, paracrine and neurocrine roles of the orexin/OXR system in the peripheral nervous system such as hypothalamus–pituitary axis (HPA) and gastrointestinal tract are reviewed by Voisin et al. and Heinonen et al. [44,45] (Fig. 1). However, reports of peripheral orexin should be treated with caution since an orexin-like peptide, which cross-reacts with orexin antisera, may be a source of confusion [46].

3. The orexin signaling pathways

The intracellular signaling pathways that mediate the effects of orexins have been intensively investigated. The most significant effect of orexin upon cells is the depolarization of neurons leading to increased excitability and firing rate [17,61–63]. This depolarization is attained by inhibition of K+ channels or activation of nonspecific cation channels

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