Targeting the orexinergic system: Mainly but not only for sleep-wakefulness therapies

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Abstract Orexin receptors belong to the big family of G protein coupled receptors (GPCRs) that constitute the main targets in the modern pharmacological approaches. Although the orexinergic system is involved in a variety of processes, treating sleep-wakefulness disorders such as narcolepsy and insomnia, remains the main therapeutic implication of targeting orexinergic receptors. After novel advances, such as the description of the binding pockets, and ligand developments, more researchers are focusing on orexin receptors as promising targets. Furthermore, targeting these receptors may provide therapeutic solutions for some health problems, other than sleep-wakefulness disorders including some psychiatric disorders and neurodegenerative diseases. Within this paper, we put a spotlight on the orexins’ physiology, pathophysiology and pharmacology of mainly sleep-wakefulness. We have also reviewed examples about other orexinergic system-related disorders. We further illustrated recent development in orexin receptors’ agonists and antagonists. In addition, we discussed selected progresses in orexinergic receptors’ ligands.

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1. Introduction

Among the emerging targets in neuropharmacology, orexin receptors are objects of more focus. A food intake study had led, in 1998, to the discovery of neurotransmitter orexins (OX), also called hypocretins (hcrt), in the rat lateral hypothalamic area (LHA). The hypothalamic neuropeptides orexin-A and orexin-B interact with two orphan GPCRs. Indeed, orexinergic receptors (OXR) type 1 and type 2 (OX1R and OX2R) are G-protein-coupled receptors (GPCRs). Orexin-B has been pointed as showing 10–100-fold higher potency at the OX2 receptor than at the OX1 receptor, whereas OX-A binds with similar affinity to both receptors. It has also been reported that during proestrous afternoon there is an increased expression of OX1R and OX2R, and, in sleep, the secretion of orexin is seasonally dependent, which shows a time-dependant activity of the orexinergic system. Studies on the intracellular pathways and signal transductions have shown that Ox/hcrt-R1 is coupled to Gq-type G protein, whereas ox/hcrt-R2 is coupled to both Gi/o and Gq proteins. GPCRs are receptors that are characterized by the factors that can influence their activity and the signal transduction signal they implicate. Regarding the corresponding intracellular downstream, orexin G protein-coupled receptors couple to different proteins including phospholipases and kinases. The cellular and molecular bases of the orexinergic system’s functions lie on both the orexinergic receptors and the corresponding pathway that involves diverse intracellular mechanisms such as the inhibition of the cyclic AMP(cAMP) synthesis. Orexins and their receptors have been described both inside and outside the brain, for instance, the presence of both receptors in the ovary was demonstrated. Within this mini-review, we describe, via illustrative examples, some of the possible therapeutic applications and pharmacological properties that have been linked to the orexinergic system. Although treatment of sleep-wakefulness disorders, like insomnia, remains the main pharmacological application, other therapeutical implications seem interesting.

2. Physiological and pathophysiological overview of the orexinergic system

Orexin system (ox/hcrt system) has been described in several physiological and pathophysiological processes, mainly in the regulation of sleep and wakefulness. Indeed, the ventrolateral preoptic nucleus (VLPO) is suggested to send inhibitory signals to both orexin neurons and the “arousal center”; whereas orexin neurons depolarize the “arousal center” during the wake stage. Other functions and properties, among which selected examples are summarized in Table 1, have also been linked to the orexinergic system. Furthermore, since peripheral organs have been shown to involve orexins, we suggest that orexins play other roles in more physiological functions which suppose novel possibilities for the related pharmacotherapies.

3. Pharmacology and potential therapeutics

Orexinergic system-related pharmacological and therapeutic researches have recently started to overcome the struggle of the lack of developed synthetic antagonists. However, the majority of those synthetic antagonists are not commercially available. Agonist and antagonist binding pockets of orexin receptors are gradually being unveiled, therefore, we expect more advances in ligand researches. Sleep-wakefulness

Table 1  Mechanisms and effects in which the orexinergic system has been shown, or hypothesized, to play roles.

<table>
<thead>
<tr>
<th>Mechanisms/effects</th>
<th>References</th>
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<tr>
<td>Regulation of appetite and stress response</td>
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<td>Respiratory effects</td>
<td>27-29</td>
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<tr>
<td>Addiction</td>
<td>11,25,26,30-38</td>
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<tr>
<td>Behavioral changes associated with cocaine administration</td>
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<td>Analgesia</td>
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<td>Food intake regulation</td>
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<td>Reproduction</td>
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<td>Motor activity</td>
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<td>Migraine</td>
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remains the main topic among orexin system-related researches. In fact, selective activation of ox/hcrt-R2 promotes wakefulness.\textsuperscript{74} In addition, whereas orexin neuron inactivation was linked with sleep, their activation promotes the wakefulness.\textsuperscript{74,75} Therefore, pharmaceutical companies are aiming to develop agents that would target the Hcrt system \textit{in vivo}\textsuperscript{70} as new therapies, not only for sleep disorders, but also for other diseases and disorders.\textsuperscript{76} Such advances highlight the progress of the pharmaceutical industry in the pharmacological targeting of the orexinergic system. For example, orexin receptors’ blocking could constitute a treatment for insomnia.\textsuperscript{77} Following this idea, Johnson & Johnson has pointed that blockade of OX1R can reduce OX2R antagonist-induced sleep promotion in rats.\textsuperscript{78} More importantly, due to insomnia statistics and the pathologies that have been linked to this disorder, we realize that developing treatment for insomnia will constitute an important advancement in neuropsychiatry. Insomnia is a common neuropsychiatric disorder with a chronic form that affects 20% of the adult population.\textsuperscript{79} Importantly, insomnia has been linked to depression, obesity, cardiovascular disease, cancer and chronic pain.\textsuperscript{80–84} Moreover, insomnia may lead to depression\textsuperscript{85} and sleep disturbances are frequently observed in multiple sclerosis (MS) patients.\textsuperscript{86} Studies of diverse new agents and receptors have reported numerous compounds and targets for developing novel insomnia treatments, among them we mention central nervous system (CNS) histamine receptors, GABA(A), melatonin and serotonin modulators.\textsuperscript{87} However, sedative-hypnotics that target GABA receptors represent the main current therapy for insomnia.\textsuperscript{18} Starting from the hypothesis that the new compounds that are potentially effective in various types of insomnia, which is associated with some disease, can, via the alleviation of insomnia, directly result in the improvement of those diseases, we suppose that treating insomnia using novel approaches might have three main consequences: {1} Reduce the incidence of the above mentioned disorders,\textsuperscript{12} {2} improve the prognosis of insomnia-related pathologies and {3} provide an alternative to the agents that target GABA receptors and thus, avoid the side effects of such class of drugs (see: Fig. 1).

In mammals, the hypothalamus is the unique brain region orexin neurons are restricted to\textsuperscript{49,80–84,90}, but brain anatomy shows an important distribution of orexin neurons\textsuperscript{49,80–84}, and many brain regions, in addition to other extra cerebral structures, have orexin neuron fibers including the median eminence, arcuate nucleus, pituitary, olfactory bulb, cerebral cortex, thalamus subfornical organ, area postrema, hippocampus, amygdala, indusium griseum, brainstem and spinal cord\textsuperscript{49,80,91–94}, thus pharmacological benefits of targeting orexinergic neurons might be extended into those structures in the future.

Orexinergic neurons, that form a unique central orexinergic system,\textsuperscript{50} project to regions associated with different cerebral functions such as reward, learning and memory, emotion and attention.\textsuperscript{49} supposing a possible modification of these functions by orexin receptors’ ligands, either as an eventual therapeutic approach or as a side effect of orexin receptors’ ligands. Importantly, interactions of the orexinergic system with other neurotransmitters have also been reported. For instance, in anesthetized rats, the activation of orexin neurons can be obtained via a local disinhibition of neurons of perifornical region\textsuperscript{15} of the posterior hypothalamus neurons by GABA (A) receptor antagonists.\textsuperscript{53} Interestingly, the implication of orexin A and orexin B in the regulation of monoaminergic and cholinergic neuron function in wakefulness maintenance has also been reported.\textsuperscript{3} Furthermore, orexin application to the rat brain produces serotonergic and cholinergic neuron depolarization,\textsuperscript{96,97} and orexin has been shown to modify the synaptic activity of dopamine neurons.\textsuperscript{30} Moreover, a recent publication has shown that the increase of glutamatergic synaptic transmission in the ventral tegmental area (VTA) has been linked to OX2R activity.\textsuperscript{30} Finally, whereas OxA/hcrt-1N-methyl-D aspartate receptor (NMDAR)-mediated synaptic transmission of dopamine neurons in the VTA has also been reported,\textsuperscript{95,98,102} intra-VTA administration of oxA/hcrt-1 results in an increased local dopamine and glutamate release.\textsuperscript{103,104} These interactions between the orexinergic system and other neurotransmitters could be exploited either to a better understanding of the orexinergic system underlying pathways or to develop more selective drugs. At the same time such interactions can lead to complex side effects of the orexinergic receptors’ ligands.

Based on the orexin related-pharmacological properties, pharmaceutical companies are trying to develop drugs that target orexin receptors, mainly to treat sleep disorders.\textsuperscript{1} It was reported that small molecule orexin receptor antagonists can help patients suffering from insomnia by promoting sleep.\textsuperscript{105} Herein, we state some orexin receptors’ ligands that have been either pointed in clinical trials or that may be effective for

![Figure 1](image-url)  Some possible consequences of targeting orexin receptors in insomnia.
insomnia treatment. SB-334867 constituted the most often used orexinergic antagonist in the literature.\textsuperscript{106} Whereas SB-649868 constitutes an emerging antagonist in development by GlaxoSmithKline,\textsuperscript{107} a new selective and highly potent spiroperidine-based OX2R antagonist was discovered in 2011.\textsuperscript{11} On the other hand, several papers have pointed to the two orexin receptor antagonists: suvorexant and almorexant (or ACT-078573), these small molecules have, in addition to their ability to promote sleep in animals, clinical efficacy.\textsuperscript{19,108} Furthermore, for the treatment of primary insomnia, the phase III of clinical trials of suvorexant has been completed.\textsuperscript{3} Previously suvorexant, considered as the most advanced dual orexin receptor antagonist (DORA),\textsuperscript{76} was reported to induce sleep in humans\textsuperscript{109} with analogous potency toward both OX1R and OX2R.\textsuperscript{108,110} Among suvorexant, MK-6096 (a piperidine-derived) and DORA-22 that are dual orexin receptor antagonists (DORAs),\textsuperscript{18} MK-6096 and DORA-22 show a high selectivity for OX1R and OX2R therefore, have the pharmacological properties to decrease wakefulness and thus, represent potential agents for sleep/wake dysregulation treatment.\textsuperscript{111}

Previously, eszopiclone (ESZ), a cyclopyrrolone targeting GABA-A receptor,\textsuperscript{111,112} was pointed to suppress orexinergic neurons’ activity with a possible use in insomnia treatment.\textsuperscript{113-117} This datum gets its importance from the fact that many hypnotic agents target GABA-A receptor,\textsuperscript{118,119} which strengthens the theory that links orexin system to insomnia. Another class of orexin 2 antagonists, that includes 2-methyl-3-furanyl-4H-1,2,4-triazol-3-ylthioamides, has been described as well.\textsuperscript{120} Pharmacokinetics described different routes of administration of orexin receptors’ antagonists. For instance, almorexant can be administrated orally, while SB-334867 can be injected systemically\textsuperscript{121}, thus gives a clinical use flexibility of such drugs.

Since orexin receptors are subject to active drug development, more advances are emerging and many orexin receptors antagonists are mentioned in the literature.\textsuperscript{105,120} Whereas, in 2007, GlaxoSmithKline brought out data about an orexin receptor antagonist for the clinical treatment of insomnia,\textsuperscript{20} the two dual OX1R/OX2R receptor antagonists: almorexant and MK-4305 were entered in Phase-III, but almorexant was discontinued.\textsuperscript{122} Herein, we mention that pharmacokinetic interaction of almorexant with both simvastatin and atorvastatin has been recently reported.\textsuperscript{123} It has been reported that orexin receptors’ non-subtype-selective antagonists may provide agents with hypnotic properties as well.\textsuperscript{70} Importantly, it has been shown that intraperitoneal injection of OX1R antagonist can inhibit OXA-triggered food intake, feeding behavior and weight gain.\textsuperscript{123,124} In addition, the study of the role of oxA/hcrt-1 in addiction-associated behaviors might help to develop agents for the treatment of pathological stress-related behaviors.\textsuperscript{30} Researches have also given indications about the impact of the molecular structure and receptor type on the drug activity. Indeed, D-Leu15-orexin-B (A11, DL15-orexin-B) may be more selective for OX2 receptors compared to OX1 receptors.\textsuperscript{125} In addition, factors including expression level, coupling efficiency and signal pathways have been linked to agonist potency\textsuperscript{126} thus, taking into account such data will be a good contribution to drug design especially of chemical-derived drugs.

Genetic therapy has also found its place among the orexin-related therapies. Indeed, a recent study pointed that it is possible to both control cataplexy attacks and modestly improve wake maintenance by orexin gene transfer into the dorsolateral pontine neurons.\textsuperscript{127} Finally, animal experiments contribute strongly in the evaluation of potential orexin receptor ligands. For instance, orexin-2 receptor antagonist TCS-OX2-29 was reported to induce a decreased wakefulness in rats.\textsuperscript{39} In addition, the organization of the hypothalamic orexinergic system has been studied in both giraffe (Giraffa camelopardalis) and harbor porpoise (Phocoena phocoena), which belong to the mammalian order\textsuperscript{128}, thus allow a better understanding of the human orexinergic system and provide important orientations for future trials.

## 4. Perspectives

In summary, we highlight the main pharmacological properties of orexin receptors’ ligands: Antagonists have hypnotic properties and offer a new approach to insomnia treatment,\textsuperscript{129} while agonists may have benefits for hypersomnia and narcolepsy.\textsuperscript{130} Because of some struggles, such as the non-commercial availability of most of the developed antagonists, targeting orexin receptors still constitutes a new area of research. Importantly, several papers have highlighted the possible therapeutic usages of orexin receptors’ ligands in a variety of other cerebral and neurological disorders including the use of orexin receptor antagonist as cognitive enhancer in post-traumatic stress disorder (PTSD),\textsuperscript{57} eating disorder,\textsuperscript{31,132} Alzheimer’s disease (via reducing amyloid-β),\textsuperscript{133} panic anxiety\textsuperscript{134} and addiction.\textsuperscript{135,136} In addition, as orexin (hypocretin) has been linked to both nicotine withdrawal (130) and addiction,\textsuperscript{37,138} it was supposed that further investigations on this property may lead to develop agents that can help smoking cessation.\textsuperscript{139} Moreover, in tetrahydrocannabinol (THC) as well as nicotine dependence, the orexinergic system constitutes a potential target,\textsuperscript{137} which may give more importance to this emerging aspect of cerebral pharmacology. Moreover, whereas in some clinical anxiety disorders, characterized by intense emotional arousal, orexin receptor antagonists might turn out to be a therapy.\textsuperscript{140} The orexin receptor antagonist (MK-6096) has been reported as a potential treatment of chronic migraine.\textsuperscript{141} Importantly, pharmacological blockade of the orexinergic system leads to an antidepressant-like effect.\textsuperscript{69} The studied chemical properties of the potential OXR ligands may provide a starting point to future drug development through compound screening say for example. In addition, molecules, such as some laboratory reagents and solvents, that have been reported to have biological or pharmacological effects on GPCRs,\textsuperscript{142,143} could constitute candidates in drug design or experimental developments in orexinergic system-related researches. All these pharmacological and clinical data point out clearly how this new area is underestimated, thus further investigations are strongly recommended especially within the context that could clearly clarify the toxicity and pharmacology of the drugs\textsuperscript{144,145} that may be provided by natural products\textsuperscript{96,147}.

In contrast, orexinergic neuron distribution and the interactions of the orexinergic system with other neurotransmitters might result in influences of orexin receptors’ ligands on different physiological and pathophysiological mechanisms, mainly those in which orexin system is implicated. Especially if we consider the cerebral neuro-system as a complex network within which different neurotransmitters are in continuous...
interactions. Therefore, a particular pharmacovigilance for agents targeting the orexinergic system remains highly important.

Conflict of interest

The authors declare that there is no conflict of interest.

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