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Hypocretin/Orexin Receptor Pharmacology and Sleep Phases

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Abstract

The hypocretins/orexins are two excitatory neuropeptides, alternately called HCRT1 or orexin-A and HCRT2 or orexin-B, that are the endogenous ligands for two G-protein-coupled receptors, HCRTR1/OX_1R and HCRTR2/OX_2R. Shortly after the discovery of this system, degeneration of hypocretin/orexin-producing neurons was implicated in the etiology of the sleep disorder narcolepsy. The involvement of this system in a disorder characterized by the loss of control over arousal state boundaries also suggested its role as a critical component of endogenous sleep-wake regulatory circuitry. The broad projections of the hypocretin/orexin-producing neurons, along with differential expression of the two receptors in the projection fields of these neurons, suggest distinct roles for these receptors. While HCRTR1/OX_1R is associated with regulation of motivation, reward, and autonomic functions, HCRTR2/OX_2R is strongly linked to sleep-wake control. The association of hypocretin/orexin with these physiological processes has led to intense interest in the therapeutic potential of compounds targeting these receptors. Agonists and antagonists for the hypocretin/orexin receptors have shown potential for the treatment of disorders of excessive daytime somnolence and nocturnal hyperarousal, respectively, with the first antagonists approved by the US Food and Drug Administration (FDA) in 2014 and 2019 for the treatment of insomnia. These and related compounds have also been useful tools to advance hypocretin/orexin neurobiology.

Hypocretins/Orexins and Their Receptors

Discovery

In 1998, two research groups independently identified a pair of structurally similar excitatory neuropeptides [1, 2]. These neuropeptides were determined to be the endogenous ligands for two orphan G-protein-coupled receptors (GPCRs) and were named the "hypo-

cretins" by one group [1] for their restricted localization to the hypothalamus and their structural similarity to secretin, and the "orexins" by the other group [2] after the Greek word *orexis* meaning appetite, based on the increased food intake associated with intracerebroventricular (ICV) administration of the neuropeptides in rats. Shortly after the discovery of the hypocretins/orexins, dysfunction of this neurotransmitter system was implicated in animal models of the sleep disorder narcolepsy [3, 4], which subsequently led to the discovery of loss of these neurons as the etiology underlying human narcolepsy [5, 6]. The link between this neuropeptide system and a disorder of excessive sleepiness also suggested a role for this system in the regulation of arousal states [7, 8]. The implication of this system as the cause of narcolepsy and in sleep regulation led to immediate interest in the potential of agonists and antagonists targeting the hypocretin/orexin receptors for the treatment of sleep disorders.

Neuropeptides and Receptor Pharmacology

The two neuropeptides, alternately called hypocretin 1 (HCRT1) or orexin-A and hypocretin 2 (HCRT2) or orexin-B, are cleaved from the *prepro-orexin* precursor. HCRT1/orexin-A is a 2,562-Da polypeptide, 33 amino acids in length, whereas HCRT2/orexin-B is a linear 2,397-Da polypeptide, 28 amino acids in length [2]. Both peptides share a structurally similar amidated C-terminal, which is crucial for both the binding of the peptides and activation of the cognate receptors, whereas the N-terminal peptide sequence is more variable between HCRT1/orexin-A and HCRT2/orexin-B [2]. Hypocretin/orexin receptor 1 (HCRTR1/OX_1R) and receptor 2 (HCRTR2/OX_2R) share 64% sequence similarity in humans [2]. While HCRTR1/OX_1R shows a selective binding affinity for HCRT1/orexin-A, HCRTR2/OX_2R has an equal affinity for both neuropeptides [2]. Despite the similarities of both hypocretin/orexin neuropeptide and receptor subtypes, the two receptors are differentially expressed within the brain [9], suggesting they have distinct physiological functions (Fig. 1).

GPCRs such as HCRTR1/OX_1R and HCRTR2/OX_2R are composed of seven transmembrane helices. The C-terminal portion of helices 2–4 of HCRTR1/OX_1R and HCRTR2/OX_2R confer differential selectivity of the two receptors [10], although the specific differences in the structure of HCRTR1/OX_1R and HCRTR2/OX_2R that result in receptor specificity are subtle. Both receptors also contain an N-terminal amphipathic α-helix that mediates the binding of the orexin neuropeptides [11]. A substitution of threonine for alanine at the 3.33 position and threonine for serine at the 2.61 position within the binding pocket of human HCRTR2/OX_2R causes a 5% decrease in the volume of the pocket compared with HCRTR1/OX_1R, a feature that may mediate receptor selectivity and, furthermore, could be exploited to confer specificity when designing molecules selective for each of the two receptors [11].

Evolutionary Insights

Hypocretin/orexin-containing neurons have been identified in the hypothalamic region of a variety of vertebrates, and the components of the orexin system are highly conserved

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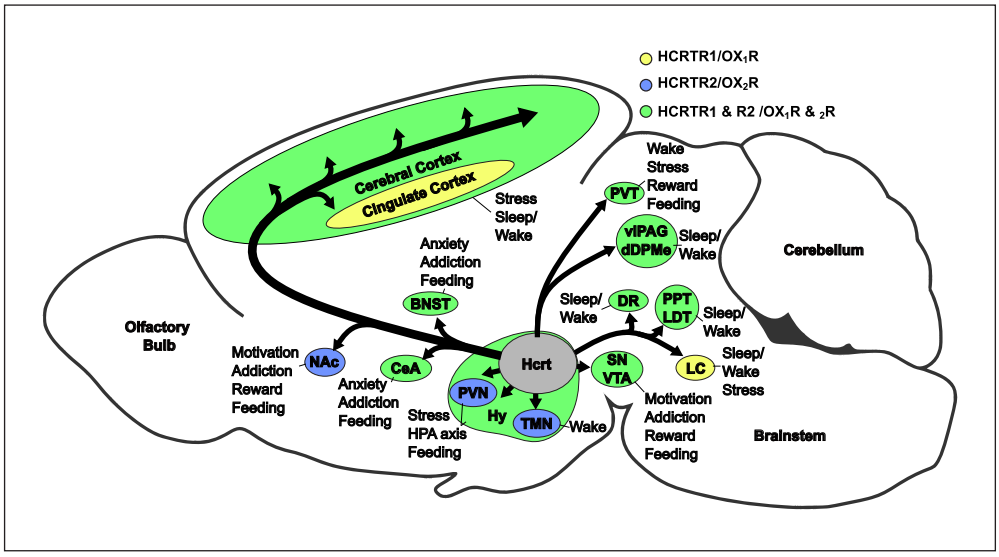

Fig. 1. Hypocretin/orexin-containing neurons and their projections, receptor distributions, and downstream behavioral outcomes. Hypocretin/orexin-producing neurons are located in the hypothalamus (Hy) and project to diverse brain regions differentially expressing hypocretin/orexin receptors 1 and 2 (OX_1R and OX_2R; receptor expression is indicated by the background color of each brain region), including the cerebral cortex, cingulate cortex, bed nucleus of the stria terminalis (BNST), nucleus accumbens (NAc), tuberomammillary nucleus (TMN), dorsal raphe (DR), locus coeruleus (LC), paraventricular thalamus (PVT), ventrolateral periaqueductal gray (vlPAG), the dorsal deep mesencephalic nucleus (dDPMe), pedunculopontine and laterodorsal tegmental nuclei (PPT and LDT), substantia nigra (SN), ventral tegmental area (VTA), central area of the amygdala (CeA), and the paraventricular nucleus (PVN). Through these projections, hypocretin/orexin-containing neurons are able to elicit diverse behavioral outputs.

across vertebrates [12]. Two genes encoding peptides with structural similarity to HCRT1/orexin-A have been identified in *Amphioxus*, an extant member of the basal chordate subphylum Cephalochordata, that is often studied to provide insight into early vertebrate evolution [13]. The orexin-A-like peptide is expressed in the neural chord of *Amphioxus*. Since the neural chord is believed to be a precursor to the vertebrate brain, the hypocretin/orexin system might have already been present in some form prior to the diversification of the vertebrates. The presence of a conserved hypocretin/orexin neuronal system among widespread phylogenetic groups indicates strong selective pressure to maintain a vital function subserved by this network.

Hypocretin/Orexin Receptor Antagonists (aka SORAs and DORAs)

Hypocretin/orexin-producing neurons project to brain regions involved in regulating reward, learning, memory, emotion, attention, and arousal states [14]. The innervation of brain structures involved in the regulation of these various functions has made the development of hypocretin/orexin receptor antagonists an active area of investigation for the treatment of addiction, sleep disorders, obesity, mood, anxiety, and panic disorders. There are two main classes of hypocretin/orexin receptor antagonists, those selective for a spe-

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Table 1. Effects of hypocretin/orexin receptor agonists and antagonists on sleep/wake in preclinical studies

Drugs	Species/strain	Arousal state parameters				Ref.
		wakefulness	NREM sleep	REM sleep	cataplexy	
Hypocretin/orexin receptor agonists						
HCRT1/orexin-A (dual OX_1R/OX_2R agonist)	WT, *OX_1R* KO, *OX_2R* KO and *OX_1R;OX_2R* DKO mice	↑	↓	↓	N/A	[42]
TAK925 (HCRTR2/OX_2R agonist)	C57BL/6J, *OX_2R* KO and *orexin/ataxin-3* mice	↑	↓	↓	↓	[63, 65]
TAK994 (HCRTR2/OX_2R agonist)	*Orexin/ataxin-3* mice	↑	↓	↓	N/R	[69]
YNT185 (HCRTR2/OX_2R agonist)	C57BL/*6J*, *prepro-OXKO*, and *OX_1R;OX_2R* DKO and *orexin/ataxin-3* mice	↑	↓	ICV: NC; IP: ↓	↓	[62]
Dual hypocretin/orexin receptor antagonists (DORAs)						
Almorexant (ACT-078573)	Wistar rats	↓	↑	↑	N/A	[15]
	Sprague-Dawley rats	↓	↑	↑	N/A	[74]
	Sprague-Dawley rats	↓	↑	↑	N/A	[48]
	Orexin/ataxin-3 and WT littermate mice	↓	↑	↑	↑	[86]
	C57BL/6NCrlCrlj mice, F344/DuCrlCrlj rats, Sprague-Dawley rats, *orexin/ataxin-3* mice	↓	↑	↑	N/R	[87]
Daridorexant (ACT-541468)	Wistar rats	↓	↑	↑	N/R	[29]
Lemborexant (E2006)	C57BL/6NCrlCrlj mice, F344/DuCrlCrlj rats, Sprague-Dawley rats, *orexin/ataxin-3* mice	↓	↑	↑	N/R	[87]
	C57BL/6J and *prepro-OXKO* mice	↓	↑	↑	↑	[88]
Suvorexant (MK-4305)	Sprague-Dawley rats	↓	↑	↑	N/A	[89]
	Sprague-Dawley rats	↓	↑	↑	N/A	[20]
HCRTR1/OX_1R antagonists (1-SORAs)						
ACT-335827	Sprague-Dawley rats	NC	NC	NC	N/A	[50]
Compound 56	Sprague-Dawley rats	NC	NC	NC	N/A	[90]
	OX WT mice	N/R	NC	NC	N/A	[90]
	OX_2R KO mice	N/R	NC	↑	N/R	[90]
GSK-1059865	Sprague-Dawley rats	NC	NC	NC	N/A	[91]
JNJ-54717793	Sprague-Dawley rats	N/R	NC	NC	N/A	[38]
	OX WT mice	N/R	NC	NC	N/A	[38]
	OX_2R KO mice	N/R	NC	↑	N/R	[38]
JNJ-61393215	Sprague-Dawley rats	NC	NC	NC	N/A	[55]
	OX WT mice	N/R	NC	NC	N/A	[55]
	OX_2R KO mice	N/R	NC	↑	N/R	[55]
SB-334867	Sprague-Dawley rats	↓	↑	↑	N/A	[48]
	Hooded Lister rats	NC	NC	NC	N/A	[49]

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Table 1 (continued)

Drugs	Species/strain	Arousal state parameters				Ref.
		wakefulness	NREM sleep	REM sleep	cataplexy	
HCRTR2/OX_2R antagonists (2-SORAs)						
EMPA	Sprague-Dawley rats	NC (most doses)	↑ (high dose only)	NC	N/A	[48]
JNJ-10397049	Sprague-Dawley rats	N/R	↑	NC	N/A	[74]
MK-1064	WT and HCRTR1; HCRTR2 DKO mice, Sprague-Dawley rats, and beagles	↓	↑	NC in mice; ↑ in rat	N/R in DKO mice; NC in beagles	[84]
MK-3697	Mouse, rat, and dog	↓	↑	↑	N/A	[75]
MK-8133	Mouse, rat, and dog	↓	↑	↑ in rat only	N/A	[92]
Seltorexant (JNJ-42847922/ MIN-202)	Sprague-Dawley rats, C57BL6 and OX_2R KO mice	N/R	↑	NC	N/R	[73]

DKO, double knockout; HCRT, hypocretin; HCRTR, hypocretin receptor; ICV, intracerebroventricular; IP, intraperitoneal; KO, knockout; N/A, not applicable; NC, no change; N/R, not reported; NREM, non-rapid eye movement; REM, rapid eye movement; WT, wild-type.

cific hypocretin/orexin receptor known as selective orexin receptor antagonists (SORAs), i.e. selective antagonists for receptor 1 (1-SORAs) or receptor 2 (2-SORAs), and those with binding affinity for both receptors, the dual orexin receptor antagonists (DORAs). Most of the current hypocretin/orexin receptor-directed drugs have focused on antagonizing the role of this system in promoting and maintaining wakefulness for the treatment of insomnia and other disorders of nocturnal sleep disruption.

Hypothalamic hypocretin/orexin-producing neurons promote wakefulness through release of these excitatory peptides and glutamate at synapses in multiple brain regions involved in the regulation of arousal states (Fig. 1). Conversely, hypocretin/orexin antagonists promote sleep through the inhibition of the waking drive mediated by the hypocretin/orexin system; consequently, these drugs are used for the treatment of insomnia and disorders of impaired nocturnal sleep. Two DORAs, suvorexant and lemborexant, have been approved by the US Food and Drug Administration (FDA) to date, and a number are in clinical trials. Although almorexant was the first DORA shown to reduce sleep latency in several species (Tables 1 and 2) and to decrease wake after sleep onset (WASO) in humans [15, 16], clinical development was curtailed due to its hepatoxicity.

In 2014, the DORA suvorexant (formerly, MK-4305) was the first hypocretin/orexin antagonist approved by the FDA for the treatment of chronic insomnia. Suvorexant binds to HCRTR1/OX_1R and HCRTR2/OX_2R with nanomolar affinity, thereby inhibiting hypocretin/orexin receptor signaling through the competitive occupation of receptor binding sites [17]. The binding of suvorexant to HCRTR2/OX_2R also stabilizes a network of extracellular salt bridges and blocks transmembrane helix motions needed for receptor activation [18]. This inhibition of orexin receptor signaling promotes sleep by attenuating the

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Table 2. Effects of hypocretin/orexin receptor antagonists on sleep/wake parameters in clinical studies

Drugs	Subject population	WASO	TST	SOL	SWSL	NREM/ SWS	REM	REML	LPS	SE	Ref.
DORAs											
Almorexant	Healthy control samples	NC	NC	N/R	↓	NC	↑	↓	↓	NC	[15, 93]
	Adult chronic insomnia samples (≥100 mg)	↓	↑	↓	↓	↑	↑	↓	↓	↑	[93]
	Elderly patients with primary insomnia	↓	↑	N/R	NC	NC	↑	↓	↓	N/R	[94]
Daridorexant	Insomnia disorder	↓	↑	N/R	N/R	N/R	N/R	N/R	↓	N/R	[27]
	Elderly with Insomnia	↓	↑	N/R	N/R	N/R	N/R	N/R	↓	N/R	[28]
Filorexant	Clinical insomnia samples	↓	↑	N/R	N/R	NC	↑	↓	↓	↑	[31]
Lemborexant	Clinical insomnia samples	↓	N/R	N/R	N/R	N/R	NC	N/R	↓	↑	[23, 24]
SB-649868	Healthy control samples	↓	↑	N/R	N/R	↓	↑	↓	↓	N/R	[95]
	Healthy control sample in situational insomnia	↓	↑	N/R	N/R	↑	↑	↓	↓	↑	[96]
	Clinical insomnia samples	↓	↑	N/R	↓	↓	↑	↓	↓	↑	[97]
Suvorexant	Healthy control samples	↓	↑	N/R	N/R	↑	↑	N/R	↓	↑	[21, 98]
	Clinical insomnia samples	↓	↑	↓	N/R	↑	↑	↓	↓	↑	[82, 99]
	Insomnia with obstructive sleep apnea	↓	↑	N/R	N/R	↑	↑	N/R	NC	↑	[100]
	Chronic obstructive pulmonary disorder	↓	↑	N/R	N/R	↑	↑	N/R	↓	↑	[101]
2-SORAs											
Seltorexant	Healthy control samples	N/R	↑ (somnolence)	N/R	N/R	N/R	N/R	N/R	N/R	N/R	[102]
	Insomnia without psychiatric comorbidity	↓	↑	↓	N/R	NC(N3)	↑	↓	↓	↑	[25]
	Major depressive disorder (MDD)	NC	NC	N/R	N/R	N/R	N/R	N/R	↓	↑	[78]
	MDD with persistent insomnia	NC	↑	N/R	N/R	NC (N2 & N3)	NC	N/R	↓	↑	[76]
MK-1064	Healthy control samples	↓	↑	N/R	N/R	↑	↑	↓	↓	↑	[84]

LPS, latency to persistent sleep; N2, Stage N2 sleep; N3, Stage N3 sleep; NC, no change; N/R, not reported; NREM/SWS, non-rapid eye movement/slow-wave sleep; REM, rapid eye movement; REML, rapid eye movement sleep latency; SE, sleep efficiency; SOL, sleep onset latency; SWSL, slow-wave sleep latency; TST, total sleep time; WASO, wake after sleep onset.

wake-promoting effect of the hypocretin/orexin system, resulting in an increase in the number of transitions to non-rapid eye movement (NREM) and REM sleep in laboratory rodents whose sleep is polyphasic (Table 1) [19]. Like almorexant, suvorexant reduces the latency to persistent sleep (LPS), decreases WASO, and increases total sleep time (TST; Table 2) in humans [20–22]. The DORA lemborexant [23, 24] was approved by the FDA for insomnia in 2019 (Table 2). Several other drugs with sleep-promoting effects, for example, the 2-SORA seltorexant [25, 26] are in clinical development (Table 2) and a New Drug Application for the DORA daridorexant, which showed significant improvements in sleep as well as daytime functioning in clinical trials [27–29], was submitted to the FDA in January 2021. Development of the DORA filorexant (MK-6096) [30, 31] was abandoned by Merck.

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HCRTR1/OX_1R

Putative Roles

HCRTR1/OX_1R knockout (KO) mice exhibit mild sleep disruption, increased anxiety, depression-like behavior, and startle response, as well as decreased locomotory activity, prepulse inhibition, and social interaction, suggesting a diverse role for HCRTR1/OX_1R [32]. HCRTR1/OX_1R signaling has also been implicated in drug, alcohol, and food-seeking behavior through modulation of motivational activation (Fig. 1) [33]. Studies utilizing HCRTR1/OX_1R antagonists have described anxiolytic, anti-stress, and anti-rewarding properties, further implying a role for HCRTR1/OX_1R signaling in these processes [34–38]. Although *HCRTR2/OX_2R* and dual *OX_1R/OX_2R* null mutant (i.e., KO) mice both exhibit a sleep and behavioral phenotype consistent with narcolepsy, the phenotype is more pronounced in the dual receptor KO mice [39–41], suggesting synergistic interaction between the two hypocretin/orexin receptor subtypes in sleep-wake control.

Selective HCRTR1/OX_1R Agonists

To our knowledge, no selective HCRTR1/OX_1R agonists have been identified to date. ICV administration of HCRT1/orexin-A, the endogenous ligand for both HCRTR1/OX_1R (IC_{50} = 20 nM) and HCRTR2/OX_2R (IC_{50} = 38 nM) [2], results in increased wake and decreased NREM and REM sleep compared to control mice receiving vehicle injections [42]. When administered intranasally in humans, HCRT1/orexin-A has a REM-stabilizing effect but, given the affinity of orexin-A for both HCRTR1/OX_1R and HCRTR2/OX_2R, the effects of administration of this peptide could be mediated by either or both receptors [43, 44]. While sleep regulation is more clearly associated with HCRTR2/OX_2R agonism, HCRT1/orexin-A administration also triggers drug-seeking behavior, alterations in autonomic physiology, increased food intake, and has antidepressant effects which are likely regulated by HCRTR1/OX_1R activation [34, 45–47]. These results suggest some of the physiological effects that might be expected from HCRTR1/OX_1R agonists.

Selective HCRTR1/OX_1R Antagonists (1-SORAs)

SB-334867 was the first 1-SORA to be described [35] and has been shown to reduce reward-seeking behavior associated with drug addiction and the consumption of high-fat foods [34–37]. SB-334867 also decreases wake and increases both NREM and REM sleep in rats, an effect that is consistent with, but is modest in comparison to, that seen following the administration of DORAs (Table 1) [48]. Furthermore, SB-334867 reverses the sleep modulatory effects of HCRT1/orexin-A administration [49]. The sleep modulatory effects of 1-SORAs have not been consistent between studies and compounds; nevertheless, these results suggest a modest role for HCRTR1/OX_1R signaling in sleep regulation that will require further study to fully elucidate [48–50].

Administration of another 1-SORA, JNJ-54717793, reduced panic-induced behaviors and cardiovascular responses in preclinical models of panic and anxiety without affecting baseline activity patterns, suggesting that HCRTR1/OX_1R antagonists might represent a

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novel treatment for anxiety conditions [38]. Several other 1-SORAs have been identified; however, their behavioral effects and therapeutic utility have yet to be evaluated [51–53]. While DORAs have also shown potential in the treatment of addiction and certain neuropsychiatric conditions, 1-SORAs do not have the strong sleep-promoting effect that DORAs do, thus giving 1-SORAs a distinct advantage as potential addiction and anxiety therapeutics. Two 1-SORAs, ACT-539313 and JNJ-61393215, are in clinical testing [54, 55].

HCRTR2/OX_2R

Putative Role in Sleep/Wake

As mentioned above, HCRTR2/OX_2R has an equal affinity for both HCRT1/orexin-A and HCRT2/orexin-B [2]. HCRTR2/OX_2R is expressed primarily in the cortical layer VI, the nucleus accumbens, raphe nuclei, septal nuclei, the subthalamic and paraventricular thalamic nuclei, the anterior pretectal nucleus, and many hypothalamic nuclei including the tuberomammillary nucleus, dorsomedial nucleus, paraventricular nucleus, and premammillary nucleus (Fig. 1) [9]. Landmark studies have demonstrated that loss of orexin neurons and the resulting hypocretin/orexin deficiency is associated with narcolepsy type 1 (NT1) [5, 6, 56]. Mice lacking HCRTR2/OX_2R exhibit a narcoleptic phenotype characterized by fragmentation of sleep-wake states and cataplexy-like episodes [39], while *HCRTR1/OX_1R* KO mice only display mild fragmentation of sleep-wake cycles, with no other overt signs of narcoleptic symptomatology [41]. In the 1970s, several strains of large breed dogs were found to display an inherited narcolepsy-like phenotype characterized by cataplectic attacks, sleep fragmentation, and other sleep-wake symptoms associated with narcolepsy. This phenotype was transmitted as an autosomal recessive gene called *canarc-1* that was later determined to encode a mutation in *HCRTR2/OX_2R* [4]. Together, these studies suggested a pivotal role for HCRTR2/OX_2R in the pathophysiology of narcolepsy, at least in animal models. The implication of the loss of hypocretin/orexin-containing neurons in the etiology of the human narcolepsy further suggested a sleep-wake regulatory function for this system. While HCRTR1/OX_1R expression appears to be reduced in the brain of human narcoleptics, HCRTR2/OX_2R expression remains high [57]. Thus, the pharmacological substrate for a hypocretin/orexin therapeutic remains intact, a conclusion supported by studies demonstrating the amelioration of sleep symptoms in NT1 patients through the intranasal application of HCRT1/orexin-A [43, 44]. Consequently, HCRTR2/OX_2R agonism could be an effective therapeutic strategy to address hypocretin/orexin deficiency in NT1, although it is also possible that combined HCRTR2/OX_2R and HCRTR1/OX_1R agonism or OX_1R agonism alone might bring some benefit to narcoleptics.

Selective HCRTR2/OX_2R Agonists

Intrathecal and ICV administration of HCRT1/orexin-A increases wake and suppresses cataplexy in hypocretin/orexin neuron-ablated and hypocretin/orexin peptide-deficient mouse models of narcolepsy [58, 59] but not in HCRTR2/OX_2R-deficient narcoleptic ca-

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nines [60], suggesting the potential for selective HCRTR2/OX_2R agonists in the treatment of narcolepsy and other disorders of excessive sleepiness. The first selective nonpeptide HCRTR2/OX_2R agonist, YNT185 (EC_{50} on OX_2R = 23 nM, OX_1R/OX_2R EC_{50} ratio = 70), was described in 2015 [61]. Systemic administration of YNT185 reduced cataplectic attacks in hypocretin/orexin peptide-deficient and hypocretin/orexin neuron-ablated mice but not in hypocretin/orexin receptor-deficient mice (Table 1) [62]. These results provided a proof-of-concept for hypocretin/orexin replacement therapy with HCRTR2/OX_2R agonists for NT1. Peripherally administered YNT185 promoted wakefulness in hypocretin/orexin-deficient, hypocretin/orexin neuron-ablated and wild-type mice, suggesting that hypocretin/orexin receptor agonists may be useful for treating sleepiness due to NT1 and other causes. No rebound was observed in sleep parameters during the active phase after YNT185-induced increases in wakefulness in either wild-type or hypocretin/orexin-deficient mice; a result that should be more thoroughly investigated [62]. Ultimately though, YNT185 has limited in vivo efficacy and thus appears unsuitable for further clinical development.

Yukitake et al. [63] described the HCRTR2/OX_2R-selective agonist TAK925 (Table 1) with an EC_{50} on OX_2R = 5.5 nM and >5,000-fold selectivity over HCRTR1/OX_1R. TAK925 has modest wake-promoting effects in wild-type mice and nonhuman primates when injected subcutaneously or intravenously (Table 1) [64]. In the *orexin/ataxin-3* mouse model of narcolepsy, TAK925 increased wake and reduced sleep-wake fragmentation and cataplexy (Table 1) [65]. The wake-promoting effect of TAK925 was not diminished after 14 days subchronic administration [65]. Preliminary data also showed that TAK925 attenuated body weight gain [66], a symptom in human narcolepsy that also occurs in *orexin/ataxin-3* mice without increases in daily food intake [67]. A Phase 1 study in healthy sleep-deprived adults demonstrated that TAK925 was well-tolerated and increased wakefulness at night compared to placebo [68]. If these results are confirmed in a broader Phase 2 study and the drug is found to be safe and effective, TAK925 could become the first narcoleptic therapeutic directed toward the neurotransmitter system whose dysfunction is implicated in the etiology of the disorder. However, TAK925 requires intravenous administration, which has driven The Takeda Pharmaceutical Company to pursue development of other orally available agonists more suitable for clinical application.

Ishikawa et al. [69] also described an orally available HCRTR2/OX_2R agonist, TAK994 (Table 1), with an EC_{50} on OX_2R = 19 nM and >700-fold selectivity against OX_1R. Oral administration of TAK994 during the sleep phase at 5 h into the light period promoted wakefulness in wild-type mice, but not in *HCRTR2/OX_2R* KO mice (Table 1) [69]. Oral administration of TAK994 during the active phase in both *orexin/ataxin-3* [67] and *orexin-tTA;TetO DTA* [70] narcolepsy mouse models significantly increased time spent in wake and improved wake maintenance while suppressing cataplectic episodes [71]. TAK994 had wake-promoting effects following chronic dosing for up to 14 days in *orexin/ataxin-3* mice without causing a sleep rebound [72]. A Phase 1 trial of TAK994 in healthy volunteers has been completed.

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Selective HCRTR2/OX_2R Antagonists (2-SORAs)

Seltorexant (JNJ-42847922; MIN-202) is an orally active, high affinity, and selective HCRTR2/OX_2R antagonist under development for the treatment of patients with major depressive disorder (MDD) and insomnia. Seltorexant crosses the blood-brain barrier and quickly occupies HCRTR2/OX_2R binding sites in the rat brain [73]. In a randomized Phase 2 study to evaluate the efficacy of seltorexant in treating insomnia without psychiatric comorbidity [25], oral administration of seltorexant facilitated sleep onset and prolonged sleep duration while also improving sleep quality, as indicated by decreased WASO over the first 6 h of the night (Table 2). The improvement of these sleep parameters by seltorexant was significantly greater than zolpidem in this study [25]. Several other 2-SORAs have also shown promising sleep-promoting effects in preclinical testing (Table 1) [48, 74, 75].

Insomnia is common in patients with MDD; however, the sleep-promoting effects of 2-SORAs have not been consistent in studies of MDD patients exhibiting insomnia. A Phase 2b trial of seltorexant as adjunct to antidepressant therapy in adults with treatment-resistant MDD showed a statistically significant, dose-dependent decrease in LPS, increased TST, increased sleep efficiency, and a tendency towards subjectively improved mood (Table 2) [76, 77]. In a separate Phase 2 trial of seltorexant in MDD patients with insomnia [77], a decrease in LPS and WASO, and an increase in TST failed to reach significance. However, antidepressant efficacy was correlated significantly with increased delta-power during stage 2 sleep. Hypocretin/orexin neurons project to multiple brain regions involved in the secretion and regulation of stress hormones such as those involved in the hypothalamic-pituitary-adrenal (HPA) axis in animals (Fig. 1) (e.g., adrenocorticotropic hormone and cortisol) [78]. The HPA axis is known to be overactive in depressed patients, a significant proportion of whom suffer from insomnia. Use of 2-SORAs or DORAs for the treatment of insomnia in patients with MDD may also help stabilize the HPA axis, but both this potential action on the HPA axis and the efficacy of hypocretin/orexin antagonists for the treatment of nocturnal hyperarousal in MDD patients requires further exploration.

Conclusions and Perspective

Therapeutics targeting the hypocretin/orexin receptors for the treatment of addiction, anxiety, mood, and sleep disorders represent a relatively new principle, with just two hypocretin/orexin antagonists approved by mid-2021 by the FDA for use in humans for the treatment of insomnia. Given the range of physiological processes in which the hypocretin/orexin system has been implicated, hypocretin/orexin receptor antagonists/agonists have broad therapeutic potential. Several HCRTR2/OX_2R agonists (Table 1), most notably TAK925 and more recently TAK994, have shown promise in both preclinical and clinical studies assessing their potential as treatments for narcolepsy with hypocretin/orexin-deficiency. Both DORAs and 2-SORAs have been explored primarily for their

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sleep-promoting properties in humans, and DORAs along with 1-SORAs have shown promise in the reduction of reward in food and drug addiction paradigms in rodents (Tables 1 and 2). Polysomnographic (PSG) studies have revealed improved sleep quality and increased duration for both DORAs and 2-SORAs (Table 2) [15, 79, 80]. The clinical efficacy and safety profile of the DORA lemborexant is broadly similar to suvorexant, although the half-life of suvorexant is shorter. Lemborexant improves objective (PSG) and subjective measures of sleep onset and sleep maintenance compared with placebo (Table 2), with the most commonly reported adverse event being next-day somnolence [23]. There was no evidence of significant rebound insomnia or withdrawal effects following treatment discontinuation. To our knowledge, suvorexant and lemborexant have not been directly compared in humans. Several other hypocretin/orexin receptor antagonists including the 2-SORA seltorexant [25] are in clinical development and a New Drug Application for the DORA daridorexant was submitted in January 2021 (Table 2) [27]. DORAs have also shown promise in the treatment of nocturnal hyperarousal associated with other disorders, including insomnia that occurs during the clinical phase of Alzheimer's disease [81].

While the sleep-promoting effect of these compounds is well established (Tables 1 and 2), their influence on sleep architecture is somewhat controversial, with conflicting data on how DORAs modulate specific sleep states [15, 80, 82, 83]. While it is well established that DORAs increase TST, several studies have found that this effect occurs primarily through an increase in REM sleep without a proportional increase in time spent in NREM sleep. By contrast, most studies utilizing 2-SORAs report that these compounds proportionally increase REM sleep and NREM sleep (Tables 1 and 2) [80]. Since coadministration of an HCRTR1/OX_1R antagonist with an HCRTR2/OX_2R antagonist has been shown to greatly attenuate the sleep-promoting effects of the HCRTR2/OX_2R antagonist, simultaneous inhibition of HCRTR1/OX_1R may reduce the sleep-promoting effects mediated by selective HCRTR2/OX_2R antagonism [74]; however, this is contradicted by preclinical research showing that the 2-SORA MK-1064 requires higher receptor occupancy than a DORA to achieve the same sleep-promoting effect [84].

If subsequent research supports these observations of differential effects of 2-SORAs and DORAs on sleep substates, the clinical utility of these compounds may ultimately depend on the sleep phenotype of the target population. While there is a clear advantage in increasing TST when treating insomnia, the impact of potentially increasing REM sleep without proportionally increasing NREM sleep, which is generally regarded as the more restorative sleep state, is unclear and warrants further exploration. On the other hand, this feature of DORAs could also be exploited and may ultimately prove advantageous for the treatment of disorders associated with REM sleep deficiencies such as post-traumatic stress and anxiety disorders. Currently, information regarding the effect of DORAs and 2-SORAs on human sleep architecture is very limited; thus, conclusions based on the results reported to date should be considered preliminary. Ultimately, a systematic comparison of hypocretin/orexin antagonists on the NREM/REM ratio and TST in both pathological and healthy populations is needed to more clearly establish how these compounds

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affect sleep architecture. If the reported trend for 2-SORAs to proportionally increase both REM and NREM sleep is replicated in future studies and broader clinical trials, such compounds may prove to be very advantageous in the treatment of sleep disorders such as insomnia in which the sleep disruption is not specific to a particular sleep stage [54, 85].

Key Take-Home Points

- Although the hypocretin/orexin system has been implicated in numerous physiological processes, it is most prominently associated with its role in the regulation of arousal states.
- While HCRTR1/OX_1R has been implicated in the regulation of reward, motivation, and autonomic processes, HCRTR2/OX_2R is most strongly associated with sleep-wake control.
- HCRTR2/OX_2R activation promotes wakefulness whereas HCRTR2/OX_2R antagonism promotes sleep.
- The regulatory role of the hypocretin/orexin system has made HCRTR1/OX_1R and HCRTR2/OX_2R attractive therapeutic targets and, as of mid-2021, two dual orexin receptor antagonists (DORAs) have been approved for treatment of disrupted nocturnal sleep.
- Selective HCRTR1/OX_1R and HCRTR2/OX_2R antagonists (1- and 2-SORAs) have shown potential in preclinical testing for the treatment of drug and food reward, motivation, anxiety, and insomnia.
- Hypocretin/orexin deficiency due to degeneration of the hypocretin/orexin-producing neurons underlies the sleep disorder narcolepsy and hypocretin/orexin replacement therapy through development of small molecule agonists is an active area of research in the treatment of this disorder.

Conflict of Interest Statement

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Author Contributions

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