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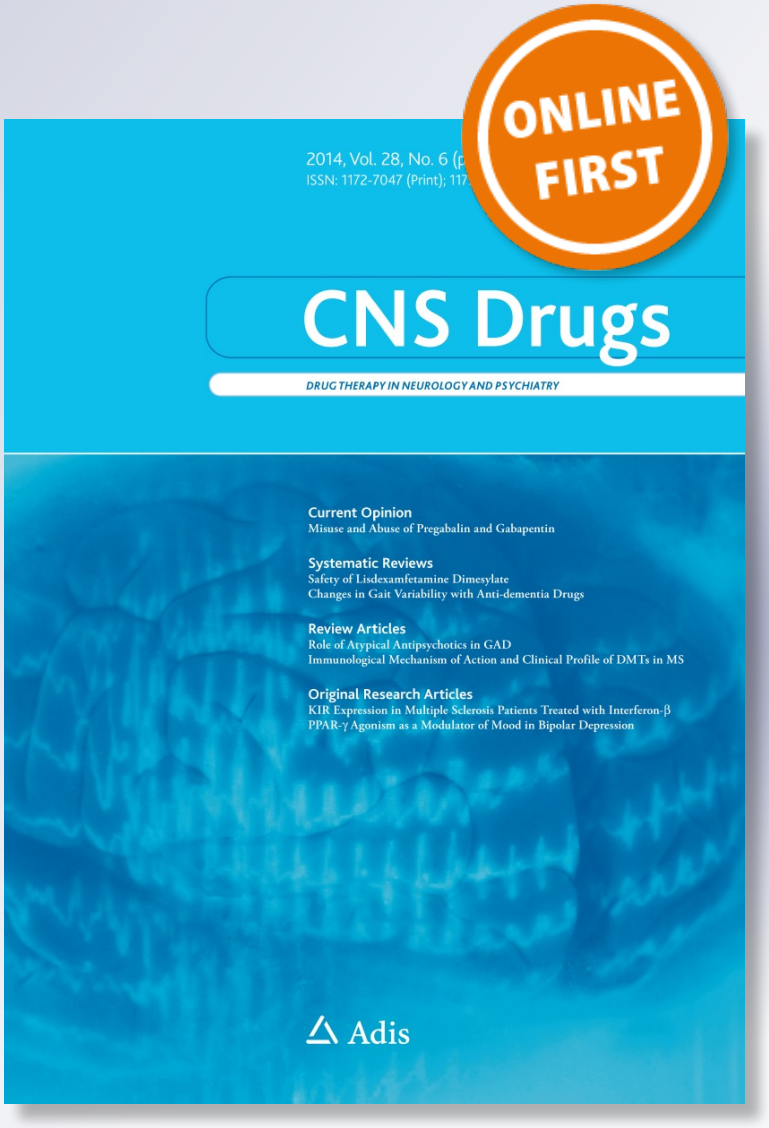
CNS Drugs

ISSN 1172-7047

CNS Drugs
DOI 10.1007/s40263-014-0179-x



Springer

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REVIEW ARTICLE

Orexin/Hypocretin Based Pharmacotherapies for the Treatment of Addiction: DORA or SORA?

Shaun Yon-Seng Khoo · Robyn Mary Brown

Abstract Addiction is a chronic relapsing disorder which presents a significant global health burden and unmet medical need. The orexin/hypocretin system is an attractive potential therapeutic target as demonstrated by the successful clinical trials of antagonist medications like Suvorexant for insomnia. It is composed of two neuropeptides, orexin-A and orexin-B and two excitatory and promiscuous G-protein coupled receptors, OX_1 and OX_2. Orexins are known to have a variety of functions, most notably in regulating arousal, appetite and reward. The orexins have been shown to have a role in mediating the effects of several drugs of abuse, such as cocaine, morphine and alcohol via projections to key brain regions such as the ventral tegmental area, nucleus accumbens and prefrontal cortex. However, it has not yet been demonstrated whether the dual orexin receptor antagonists (DORAs) under development for insomnia are ideal drugs for the treatment of addiction. The question of whether to use a DORA or single orexin receptor antagonist (SORA) for the treatment of addiction is a key question that will need to be answered in order to maximize the clinical utility of orexin receptor antagonists. This review will examine the role of the orexin/hypocretin system in addiction, orexin-based pharmacotherapies under development and factors affecting the selection of one or both orexin receptors as drug targets for the treatment of addiction.

Key Points

In preclinical animal models pharmacological antagonism of the OX_1 receptor reduces relapse-like behaviors for opiates, psychostimulants, alcohol and cannabinoids. Antagonism of the OX_2 receptor has also been shown to reduce self-administration of both opiates and alcohol.

Currently, dual orexin receptor antagonists (DORAs) are in clinical development for insomnia, with Suvorexant on the verge of approval in the United States and in pre-registration in Japan.

The selection of a DORA or a single orexin receptor antagonist (SORA) for clinical development depends on several factors, such as the specific drug of abuse and treatment aims.

1 Introduction

Addiction is a chronic relapsing disorder characterized by compulsive drug-seeking which persists despite adverse consequences [1, 2]. Use of illicit drugs, including cannabis, psychostimulants and opiates contributes 20 million or approximately 0.8 % of global Disability-Adjusted Life Years (DALYs) [3] and the use of legal drugs such as alcohol contributes another 17.6 million DALYs [4]. Relatively wealthy countries including the United States, United Kingdom, Russia and Australia are most affected and the prevalence and associated burden of addiction is increasing [3, 4]. Despite this there are relatively few

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Published online: 19 June 2014

effective and approved pharmacotherapies for addiction and these predominantly cover alcohol, nicotine and opiates [5], with topiramate for cocaine [6] and gabapentin for cannabis [7] still under development. Alcohol, determined by multicriteria decision analysis to be the most harmful drug of abuse [8], has recently had a key therapeutic, acamprosate, shown to be pharmacologically inert in animal models [9]. Demand for addiction pharmacotherapies has seen increasing 'off-label' use of baclofen in France and elsewhere [10] despite insufficient evidence to recommend its use [11]. Thus, addiction presents significant unmet medical need which invites the development of novel pharmacotherapies.

The orexin/hypocretin system is one candidate target for the development of novel pharmacotherapies that involves two neuropeptide ligands, orexin-A and orexin-B, and two G-protein coupled receptors (GPCRs), the OX_1 and OX_2 receptors. The hypocretin genes and orexin neuropeptide and receptor proteins were simultaneously discovered, and named, by two separate groups. One group was investigating genes expressed in the hypothalamus [12] and the other was investigating 'orphan' GPCRs [13]. In order to resolve the nomenclature debate the Human Genome Organization and the International Union of Basic and Clinical Pharmacology recommend that hypocretin refer to the genes and orexin refer to the gene products [14]. At present the dual orexin receptor antagonist (DORA) Suvorexant is the closest to receiving approval from the United States Food and Drug Administration (FDA) and Japan's Pharmaceutical and Medical Devices Agency (PMDA) for the treatment of insomnia so it may be possible that it finds an additional use as an anti-craving medication. However, given the weight of preclinical evidence from animal studies implicating the OX_1 receptor in addiction, which this review will discuss, it is worth considering whether a single orexin receptor antagonist (SORA) is worth developing for clinical use. This review will examine the role of the orexin/hypocretin system in addiction, orexin-based pharmacotherapies under development and factors affecting the selection of one or both orexin receptors as drug targets.

2 Pharmacology

The hypocretin gene (Human: HCRT; Rat/Mouse: *Hcrt*) is expressed exclusively in the hypothalamus, where hypocretin mRNA is translated to the prepro-orexin precursor peptide. Prepro-orexin is then cleaved to produce the 33 amino acid orexin-A peptide or the 28 amino acid orexin-B peptide [15]. Orexin-A is non-selective for the OX_1 and OX_2 receptors (encoded by HCRT1/*Hcrt1* and HCRT2/*Hcrt2* respectively), while orexin-B is approximately 10 times more selective for the OX_2 receptor [13]. Orexin-A is predicted to exist as two helices at roughly right angles that docks between transmembrane domains 3 and 5–7 [16]. The OX_1 receptor has been shown to exist primarily as a homodimer [17] but also forms complexes with other receptors, including the OX_2 receptor [18] and the cannabinoid CB_1 receptor [19]. Orexin neurons also contain a variety of cotransmitters; more than 90 % contain dynorphin [20], more than 80 % contain neurotensin [21], approximately 60 % are glutamatergic [22, 23] and some may be GABAergic [24] although evidence for GABAergic cotransmission is mixed [22].

2.1 Signal Transduction

The receptors are both excitatory [12, 13] and promiscuous, with multiple signal transduction mechanisms (Fig. 1) that vary based on tissue, agonist and agonist concentration. Both the OX_1 and OX_2 receptors rely heavily on extracellular Ca^{2+} influx, which has been shown using transfected Chinese hamster ovary, BIM (a human neuroblastoma/rat nerve-like cell hybrid) and Neuro-2a cell lines [25–27]. The release of Ca^{2+} from intracellular stores plays a role [28, 29] which suggests involvement of G_q signalling, but higher concentrations of orexin-A are required to induce release from intracellular stores than extracellular influx [30]. Influx of extracellular Ca^{2+} is also important for activation of second messengers because it increases inositol phosphate mobilisation [30, 31] suggesting that it is necessary for phospholipase C (PLC) activation. OX_1 receptors may promote Ca^{2+} influx by activating diacylglycerol-dependent cation channels, specifically the transient receptor potential canonical cation channel subfamily C members 3 and 6 (TRPC3 and TRPC6) [32, 33] and phospholipase D (PLD) via protein kinase C (PKC) [34], which implies G_q activity. Orexin appears to mobilize the endocannabinoids arachidonic acid and 2-arachidonyl glycerol (2-AG) via the phospholipase A_2 (PLA_2) and PLC-diacylglycerol lipase (DGL) pathways respectively, but these mechanisms are not yet fully understood [35, 36]. In addition to G_q and Ca^{2+}-mediated signalling, immunoprecipitation studies in OX_1-FLAG-transfected human embryonic kidney (HEK) cells demonstrate OX_1 receptors associate with G_s and G_i signalling pathways [29]. This is supported by functional studies demonstrating inhibition of cyclic adenosine monophosphate (cAMP) production at nanomolar concentrations of orexin-A is sensitive to pertussis toxin (PTX), but is eclipsed by stimulation of adenylyl cyclase at higher concentrations [26].

The OX_2 receptor is less well characterized, but has also been shown to couple to G_q, G_s and G_i and G-protein independent pathways. A dominant-negative of each G_α subunit disrupts ERK1/2 signalling in transfected HEK

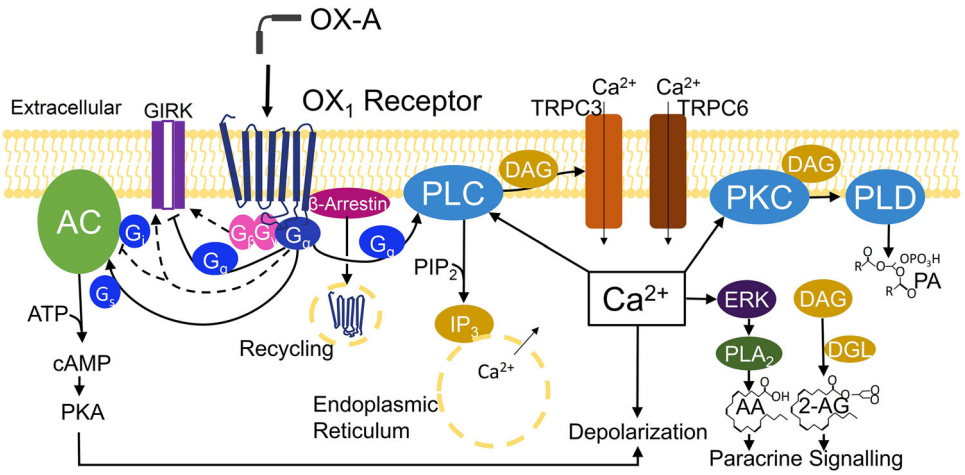

Fig. 1 Orexin receptor signal transduction. When the OX_1 receptor is activated by its ligand orexin-A, multiple signalling cascades are initiated. The initial G_i and $G_{\beta\gamma}$ activity at adenylyl cyclase and GIRKs is represented by *dashed lines*. The OX_2 receptor utilizes the same signal transduction pathways as the OX_1 receptor, but is less well characterized. *2-AG* 2-arachidonyl glycerol, *AA* arachidonic acid, *AC* adenylyl cyclase, *ATP* adenosine triphosphate, *cAMP* cyclic adenosine monophosphate, *DAG* diacylglycerol, *DGL* diacylglycerol lipase, *ERK* extracellular signal regulated kinase, *GIRK* G-protein coupled inwardly rectifying potassium channel, *IP_3* inositol triphosphate, *OX-A* orexin-A, *PA* phosphatidic acid, *PIP_2* phosphatidylinositol bisphosphate, *PKA* protein kinase A, *PKC* protein kinase C, *PLA_2* phospholipase A_2, *PLC* phospholipase C, *PLD* phospholipase D, *TRPC3* transient receptor potential canonical cation channel subfamily C member 3, *TRPC6* transient receptor potential cation channel subfamily C member 6

cells [37] and orexin-A increases radiolabelled G_s and G_i in human fetal adrenals expressing HCRT2 but not HCRT1 [38]. Further evidence for the paradoxical coupling of an excitatory receptor to the G_i pathway is provided by the PTX-sensitivity of orexin-A inhibition of Forskolin-induced cAMP production in transfected BIM cells and cultured embryonic cortical neurons [27, 39]. G_i signalling in both receptors is also partially responsible for an initial increase in G-protein coupled inwardly rectifying potassium channel (GIRK) signalling in response to orexin-A application, which is then followed by a inhibition of GIRKs [40]. Both receptors also signal through the β-arrestins, but the OX_2 receptor is more strongly biased towards β-arrestin signalling and consequently is recycled to the membrane more slowly after internalization [41, 42]. The variation in transduction mechanisms between tissues and ligands may provide future opportunities for more specific modulation of orexin signalling, but its complexity also poses a challenge.

2.2 Synthetic Orexin Ligands

Several companies have shown great interest in developing ligands for the orexin receptors, especially small organic antagonists (Table 1). There was some early development of truncated and modified orexin peptides [43, 44], such as OXA (17–33) which is a partial agonist at the OX_1 receptor [45]. Substitution of two residues in the orexin-B peptide produces [Ala^{11},D-Leu^{15}]-Orexin B (SB-668875), which is a potent agonist with increased selectivity for the OX_2 receptor [46, 47]. However, the most widely used compounds in research and all of the clinically trialled compounds are small molecule antagonists. The first was SB-334867, a naphthyridine-substituted biarylurea which is selective for the OX_1 receptor and 100 times more selective for OX_1 receptors than 50 other GPCRs and ion channels [48]. Although SB-334867 is selective for OX_1 receptors, it is still able to inhibit OX_2 receptor activity and its 50-fold selectivity for OX_1 receptors is relatively modest compared to more recently developed antagonists [48, 49].

GlaxoSmithKline has developed several commercially available small molecule OX_1 receptor antagonists. In addition to SB-334867, GlaxoSmithKline also developed SB-408124 and SB-674042 [50]. SB-408124 is urea-based, like SB-334867, although not as commonly used despite its slightly improved potency. SB-408124 has a dissociation constant (K_b) of 21.7 nM for the OX_1 receptor compared to 27.8 nM for SB-334867. SB-674042, in contrast to the two ureas, is a ketone with three heterocyclic rings and two phenyl groups [50]. It is significantly more potent than both SB-334867 and SB-408124, with a K_b of 1.1 nM for the OX_1 receptor and 129 nM for the OX_2 receptor, which also makes it a more selective OX_1 receptor antagonist than either of its predecessors [50]. More recently, Actelion Pharmaceuticals, which partnered with GlaxoSmithKline in the clinical development of Almorexant (ACT-078573) has reported a tetrahydropapaverine derivative OX_1 receptor antagonist designated ACT-335827 [51]. It is approximately 10 times more selective for the OX_1 receptor than the OX_2 receptor, with K_b values of 41 and 560 nM respectively [51].

Table 1 Synthetic orexin ligands in preclinical and clinical development

Ligand	Company	Description	References
OXA (17-33)	SmithKline Beecham	Truncated orexin-A peptide with reduced potency but enhanced selectivity for the OX_1 receptor. Partial agonist	[43]
[Ala^{11},D-Leu^{15}]-Orexin B (SB-668875)	Banyu	Substituted orexin-B peptide with enhanced selectivity for the OX_2 receptor. Agonist	[46]
SB-334867	GlaxoSmithKline	SORA. MW 319.11. Research only. First selective OX_1 receptor antagonist	[48]
SB-408124	GlaxoSmithKline	SORA. MW 356.14. Research only. Selective OX_1 receptor antagonist	[50]
SB-674042	GlaxoSmithKline	SORA. MW 448.51. Research only. Selective OX_1 receptor antagonist	[50]
ACT-335827	Actelion	SORA. MW 518.64. Research only. Selective OX_1 receptor antagonist	[51]
TCS OX2 29	Banyu	SORA. MW 397.24. Research only. First small molecule selective OX_2 receptor antagonist	[53]
JNJ-10397049	Johnson and Johnson	SORA. MW 481.98. Research only. Selective OX_2 receptor antagonist	[54]
EMPA	Roche	SORA. MW 510.23. Research only. Selective OX_2 receptor antagonist	[55]
LSN2424100	Eli Lilly	SORA. MW 407.42. Research only. Selective OX_2 receptor antagonist	[56]
MK-1064	Merck	SORA. MW 461.85. Clinical candidate. Selective OX_2 receptor antagonist	[69]
Almorexant (ACT-078573)	Actelion and GlaxoSmithKline	DORA. MW 512.23. Treatment for insomnia. Reached Phase III trials before development was discontinued	[57]
SB-649868	GlaxoSmithKline	DORA. MW 477.15. Treatment for insomnia. Reached Phase II trials before development was discontinued	[66]
Filorexant (MK-6096)	Merck	DORA. MW 420.2. Treatment for insomnia. Currently under development (Phase II). No active clinical trials	[70]
Suvorexant (MK-4305)	Merck	DORA. MW 450.16. Treatment for insomnia. Phase III trials complete, awaiting FDA and PMDA approval. Proposed addition to Schedule IV by the United States Drug Enforcement Agency	[77]

MW molecular weight

It is orally bioavailable, able to cross the blood brain barrier and has been shown in rats to reduce social interaction stress, but not diet-induced obesity [51, 52].

The small molecule selective OX_2 receptor antagonists, in contrast, were developed by a different group of companies at different times and have radically different structures. The first, TCS OX2 29, is a tetrahydroisoquinoline reported in 2003 and developed in Japan by Banyu Pharmaceutical Co., a subsidiary of Merck Sharpe & Dohme (Merck). It is highly selective for the OX_2 receptor with an IC_{50} of 40 nM while having no effect at the OX_1 receptor into the micromolar range [53]. TCS OX2 29 was soon followed by JNJ-10397049, a phenyl-dioxanyl urea compound with 600-fold selectivity for the OX_2 receptor developed by Johnson and Johnson in the United States [54]. In 2013 EMPA was described by scientists working at F. Hoffman-La Roche in Switzerland. EMPA is an acetamide with a branched structure, where each sulfonamide side chain contains either a pyridine or toluene group. It inhibits OX_2 receptor responses to orexin-A and orexin-B in the nanomolar range, but fails to inhibit OX_1 responses until beyond micromolar concentrations [55]. Eli Lilly has also recently reported the development of LSN2424100, a sulfonamide with fluorobenzene, imidazole and biphenyl side chains [56]. LSN2424100 has approximately 200-fold selectivity for the human OX_2 receptor (K_b 0.44 nM) over the OX_1 receptor (K_b 90.3 nM) and has been shown to have antidepressant-like activity in rats and mice [56].

Several DORAs have entered clinical development, including Almorexant, SB-649868, Filorexant (MK-6096) and Suvorexant (MK-4305). Almorexant, like TCS OX2 29, is a tetrahydroisoquinoline, but is a slightly larger molecule developed for the treatment of insomnia [57–60]. It has been predicted to interact with both receptors' transmembrane domains 3 and 5–7, the same domains predicted to be the site of endogenous ligand binding [61]. Although its development was discontinued in 2011 by Actelion and GlaxoSmithKline due to effects on liver enzyme parameters [62–64], it continues to be used in research and medicinal chemists are continuing to develop substituted tetrahydroisoquinolines to produce selective OX_1 receptor antagonists [65]. GlaxoSmithKline's SB-649868 is a piperidine but its carboxamide benzofuran group is instrumental for its activity [66]. Following evidence of sleep-promoting effects in rats [66], its tolerability and favourable 3–6 h half-life was demonstrated in Phase I clinical trials [67]. However, its development was later discontinued by GlaxoSmithKline [68].

Merck has two DORAs which have reached clinical trials for the treatment of insomnia, Filorexant and

Suvorexant, and has reported developing a SORA (MK-1064) for insomnia as well [69]. Filorexant is a pyridl piperidine which has sleep promoting effects in rats, mice and dogs [70, 71]. While there are few published studies of Filorexant, Merck has completed clinical trials using Filorexant to treat insomnia [72], migraine [73] and painful diabetic neuropathy [74]. However, in late 2013 Merck terminated a clinical trial of Filorexant as an adjunctive therapy for depression [75] and no longer reports MK-6096 in its pipeline updates. If there is a hiatus in Filorexant development it may be due to Suvorexant which has completed Phase III clinical trials and is awaiting FDA and PMDA approval for the treatment of insomnia [76]. Suvorexant is a substituted diazepan which has sleep-promoting effects in rats [77], dogs and rhesus monkeys [78]. In clinical trials it was found to be efficacious in promoting sleep without next-day residual effects [79, 80] and although the FDA has concerns about high-doses, it looks set to enter approved clinical use [76].

2.3 Distribution of Orexin Receptors in the Brain

There are approximately 6,000 orexin neurons in the rat [81] and 70–80,000 in humans [82, 83]. They originate in the lateral, dorsomedial and perifornical hypothalamus and project widely throughout the brain, including to key mesocorticolimbic regions implicated in reward where there is significant overlap with dopaminergic fibers [84–88]. The OX_1 and OX_2 receptors are differentially distributed within mesocorticolimbic circuitry, which suggests some functional heterogeneity [89]. It has been shown that the prefrontal cortex expresses *Hcrt1* mRNA but not *Hcrt2*, which is supported by immunohistochemical staining for OX_1 but not OX_2 receptors [89–92]. In the nucleus accumbens it is *Hcrt2* mRNA and the OX_2 receptor that is expressed [89–92]. In other regions there is expression of both *Hcrt1* and *Hcrt2*, with approximately equal expression in the ventral tegmental area (VTA) and paraventricular thalamus (PV) and slightly greater expression of *Hcrt1* in the amygdala and bed nucleus of the stria terminalis [89]. In most hypothalamic regions, including the lateral hypothalamus, *Hcrt2* is more strongly expressed [89, 90]. The results of these early mapping studies, based mainly on in situ hybridisation, may not show the full extent of the orexin receptor expression since RT-PCR has demonstrated expression of both *Hcrt1* and *Hcrt2* in the nucleus accumbens [93]. Microinfusion of SB-334867 into the nucleus accumbens has been shown to attenuate orexin-A induced feeding [94] and although higher doses of SB-334867 may also inhibit OX_2-mediated responses [49] it is unlikely that the 6 ng dose used would have had significant off-target effects. The distribution of orexin receptors may therefore be broader than currently thought.

3 Functions of the Orexin System

The orexin system modulates arousal, appetite and reward. It was originally shown that central administration of either orexin-A or orexin-B stimulated feeding in sated rats [13]. The importance of orexins for arousal has been of particular interest for research into narcolepsy; human patients with narcolepsy have approximately 90 % fewer orexin neurons than healthy individuals, demonstrating the importance of orexins for arousal [82]. Activation of orexin neurons increases wakefulness, while inhibition decreases wakefulness [95]. Orexins also coordinate goal-directed arousal, such as increased wakefulness following food restriction [96] or the anticipatory arousal associated with feeding time in food restricted mice [97]. This has been shown to extend to male sexual behavior because orexin neurons are activated during copulation and systemic administration of SB-334867 in rats has been shown to increase latency to intromission [98]. If there is a pathophysiological state of orexin-mediated arousal directed towards maladaptive reward-seeking, rather than natural rewards (such as food and sex), then it may be possible for this to be modulated with pharmacotherapies.

3.1 Pathophysiology in Relation to Addiction

The lateral hypothalamic origin of orexin neurons and their wide projections to various parts of the brain, including mesocorticolimbic regions, suggests a possible role in reward-seeking and addiction thus intensive investigation in this area followed their discovery. Indeed, much evidence now exists supporting a role for orexins in mediating central effects of multiple drugs of abuse as well as drug-seeking behavior. The first evidence came in 2003, when it was shown that morphine withdrawal activates orexin neurons and that orexin knock-out mice exhibit reduced withdrawal symptoms [99]. Systemic administration of the OX_1 receptor antagonist SB-334867 has since been shown to reduce morphine withdrawal symptoms, such as sniffing and tremor, implicating the OX_1 receptor in mediating this effect [100]. The first evidence of a role for orexins in drug reward was provided in 2005, when it was shown that activation of lateral hypothalamic orexin neurons is strongly associated with preference for contexts associated with drug reward (morphine or cocaine). Moreover, stimulation of these cell bodies was shown to reinstate an extinguished morphine conditioned place preference (CPP; see Table 2 for a summary of behavioral models), and this was blocked by administration of SB-334867. Reinstatement could also be precipitated by microinjection of orexin-A into the VTA, suggesting the VTA as a possible locus for this effect [101]. The VTA's dopaminergic projections are important for reward [102] and several studies

Table 2 Summary of preclinical behavioral models of addictive behavior

Model	Description
Conditioned place preference (CPP)	Based on Pavlovian principles, an animal is trained to associate a context (conditioned stimulus, CS) with the effects of a vehicle or drug injection (unconditioned stimulus, US). Two adjoining chambers are used to provide distinct contexts. During training the animal is confined to each context for a period of time immediately following an injection of the paired substance (vehicle or drug). On test, the animal is allowed to move freely between the two contexts and a preference for one side may suggest that the substance produced a positive internal state, thus eliciting conditioned approach towards that particular context (or conversely, that the other substance produced an aversive state)
Operant self-administration	Animals may also be trained to voluntarily acquire and ingest a drug. In the operant self-administration model an animal performs a behavior such as a lever press or nosepoke which is reinforced by the delivery of a reward, such as a drop of alcohol or intravenous bolus of cocaine
Fixed-ratio schedules	Operant self-administration may be subject to varying schedules of reinforcement. Fixed-ratio schedules model consummatory behavior and refer to a fixed number of operant responses being required for each reward delivery. For example, an FR3 schedule requires 3 lever presses for each reward delivery. FR1 is also referred to as continuous reinforcement because every operant response is reinforced
Progressive ratio schedules	A progressive ratio schedule requires an animal to make an exponentially increasing number of responses for each reward delivery. The highest number of responses that an animal will make in exchange for reward is the breakpoint. Breakpoints indicate the level of motivation an animal has for a reward
Extinction	Extinction involves new learning[a] that inhibits the CS and US association in a Pavlovian conditioning paradigm such as CPP or an action (e.g. lever press) and outcome (e.g. drug reinforcement) association as is measured in the operant self-administration paradigm. It generally involves removal of the drug or reward. In the context of CPP, the animal may be given free access to both contexts without vehicle or drug injections or administered vehicle injection in the previously drug-paired side. Over time, a preference for the drug-paired side will diminish (i.e. extinguish). In operant paradigms, the animal might be able to perform the action, but no rewards will be delivered. Over time, the number of responses the animal emits will diminish and the behavior is extinguished
Reinstatement	Reinstatement involves an increase in drug-related preference or operant responding after a period of extinction. Reinstatement may be precipitated by a priming injection of drug, stress, presentation of drug-associated cues or reintroduction to a drug paired context (or context-induced renewal). Extinction-reinstatement paradigms are intended to model rehabilitation and relapse processes
Two-bottle free choice	Animals are given free access to both a control and a drug bottle in their home cage (e.g. water vs alcohol) such that a free choice is made to drink the substance in question. This approach models consummatory behavior without any associative or operant learning
Drinking-in-the-dark	An animal's water bottle is temporarily replaced with a bottle of alcohol in their home cage during the dark phase of their light/dark cycle. This models binge drinking during the animal's active phase

[a] There is much agreement that extinction is not erasure [210], however there is also experimental evidence which suggests that extinction causes at least partial erasure of the original memories—see [211] for review

have implicated orexins in its modulation. Orexin neurons from the lateral hypothalamus form synapses with the dendrites of dopaminergic and GABAergic neurons within the VTA [103] and orexin-A and orexin-B both increase dopamine release in the nucleus accumbens [104, 105].

3.1.1 Orexin and Opiates

The orexin system may represent a direct target of opiate drugs of abuse. Lateral hypothalamic orexin neurons express μ opioid receptors [99] which inhibit the firing of orexin neurons in response to met-enkephalin or morphine. When antagonists (naloxone or CTAP) are applied, orexinergic activity is increased [106]. Slice recordings from orexin neurons in mice given chronic morphine show reduced responses to morphine, but increased responses to antagonists [106]. The altered responses of orexin neurons following chronic morphine may explain why the expression of CPP in morphine-dependent rats is correlated with the activation of orexin neurons projecting from the lateral hypothalamus to the VTA [107]. The VTA is also implicated in reinstatement of CPP as microinjection of orexin-A into the VTA has been shown to reinstate this behavior [101].

The possibility exists that the two orexin receptors have differential roles in mediating the central effects of opiates. The OX_1 receptor seems to mediate both the reinforcing and incentive motivational properties of opiates as systemic SB-334867 administration reduces operant self-administration, progressive ratio breakpoint and cue-induced (but not drug-primed) reinstatement of heroin-seeking [108]. SB-334867 also reduces morphine withdrawal in mice, suggesting OX_1 receptor involvement in this phenomenon [109]. No definitive role for the OX_2 receptor, however, has been established in opiate-seeking as yet. One recent study shows reduced self-administration

of heroin in an extended access model with systemic administration of an OX_2 receptor antagonist suggesting that the OX_2 receptor, at least in part, may mediate the reinforcing properties of opiates [110]. Yet the DORA Almorexant does not prevent the expression of morphine CPP suggesting neither OX_1 nor OX_2 mediate the conditioned rewarding effects of opiates [111]. Almorexant does, however, reduce the expression of locomotor sensitization to morphine [111]. Thus, the role of orexins in mediating the central effects of opiates is multi-faceted, covering many aspects of addictive behavior. These effects appear to be mediated predominantly through the OX_1 receptor though involvement of OX_2 cannot be ruled out and requires further investigation.

3.1.2 Orexin and Alcohol

Preclinical studies suggest similarly broad roles for orexins in the many facets of alcohol addiction and it appears the two receptors vary in terms of their involvement in the appetitive and consummatory phases of alcohol use. The OX_1 receptor is important for operant self-administration, home-cage alcohol consumption as well as cue-induced and stress-induced reinstatement of alcohol-seeking using SB-334867 in both outbred and inbred alcohol-preferring (iP) rats [112–115], however see [116] regarding null effects of SB-408124. Moreover, this effect of SB-334867 on cue-induced alcohol-seeking is observed both immediately after extinction as well as after a protracted period of abstinence [117]. By contrast, while antagonism of the OX_2 receptor by JNJ-10397049 reduces operant self-administration and acquisition, expression and reinstatement of CPP [116], TCS OX2 29 does not reduce cue-induced reinstatement of operant alcohol-seeking in male iP rats [118]. The OX_2 receptor antagonist LSN-2424100 does, however, reduce breakpoint on a progressive ratio in female iP rats [115] and ligands of both OX_1 and OX_2 as well as the DORA Almorexant have also recently been shown to reduce 'binge drinking' in C57BL/6J mice [115]. Thus, it appears that orexin signalling via both orexin receptors mediates the primary reinforcing effects of alcohol, whereas it is the OX_1 receptor that is primarily involved in alcohol-seeking behavior.

The orexin neurons themselves appear to be a target of alcohol as acute intragastric administration reduces c-Fos activity within the perifornical region of the hypothalamus [119]. In addition, orexin neurons are activated during cue-induced reinstatement of alcohol-seeking [120]. Orexin signalling in both cortical and limbic regions also appears to be important for this behavior as c-Fos expression associated with cue-induced reinstatement is attenuated by SB-334867 specifically in the prelimbic and orbitofrontal cortices as well as nucleus accumbens core [117]. Orexin signalling in mesocorticolimbic regions also appears to underlie the reinforcing properties of alcohol as operant responding is reduced by either administration of the SORA TCS OX2 29 into the nucleus accumbens core or administration of the DORA Almorexant into the VTA [118, 121].

3.1.3 Orexin and Cocaine

In contrast to opiates and alcohol, the involvement of the orexin system in psychostimulant abuse appears to be primarily restricted to drug-seeking. Rats treated with SB-334867 show reductions in several cocaine-seeking behaviors, including operant responding following abstinence, context-induced reinstatement [122], stress-induced reinstatement [123] and cue-induced reinstatement but not self-administration [124]. SB-334867 also reduces cocaine CPP in both rats and mice [101, 125]. Glutamatergic activity in the VTA has previously been shown to be important for learning drug-related associations [126] and orexin-A potentiates glutamate postsynaptically to promote synaptic plasticity [127] while orexin-B potentiates glutamatergic signalling pre and postsynaptically [128]. These effects appear to specifically involve an interaction between AMPA receptors and orexin signalling because positive allosteric modulation of AMPA receptors using PEPA can rescue cue-induced reinstatement of cocaine-seeking after SB-334867 pretreatment [129]. Electrophysiological studies also demonstrate the ability of orexinergic signalling in the VTA to modulate inputs from the medial prefrontal cortex [130]. However, it appears that in mice cocaine-induced synaptic plasticity as measured by AMPA/NMDA receptor ratios on orexin neurons is not altered by SB-334867 [125].

The orexin system appears to selectively modulate the motivation for cocaine rewards because systemic SB-334867 can reduce the propensity of a rat to self-administer as the unit cost is increased (i.e. a reduced dose of cocaine for each lever press), under progressive ratio or FR5 conditions, but not fixed-ratio (FR1) self-administration [131, 132]. Targeted microinjection of SB-334867 to the VTA reduces progressive ratio responding, implicating VTA orexin signalling in motivation for cocaine [131]. At a cellular and molecular level, cocaine increases excitatory inputs to orexin neurons [133] and cocaine-induced postsynaptic plasticity can be facilitated in rats by orexin-A and prevented by SB-334867 [127]. Systemic SB-334867 does not affect cocaine self-administration in male or female rats, but it does attenuate cocaine-seeking during extinction and stress/cue-induced reinstatement [134]. Some sex differences are observed because SB-334867 reduces cocaine/cue-induced reinstatement in males but not females [134]. Chronic orexin antagonist administration has only received limited attention [135]. Repeated SB-334867

administration does not reduce self-administration, but when used as an adjunct to extinction it allows pretreatment to reduce cue-induced reinstatement, but only if administered prior to the reinstatement session [136]. If the orexin antagonist is administered chronically and allowed to washout prior to test, no effect is seen on CPP [111] and the magnitude of reinstatement is actually increased [136]. These findings suggest an important role for the OX_1 receptor in cocaine-seeking behavior. Further research into the possible uses of OX_1 receptor antagonists as an adjunct to behavioral therapies as well as an anti-craving medication is needed, especially in light of the complex effects of sex and chronic dosing regimens. In contrast, systemic administration of the OX_2 receptor antagonist TCS OX2 29 does not reduce operant cocaine self-administration or cue-induced reinstatement [124].

3.1.4 Orexin and Nicotine and Cannabinoids

The OX_1 receptor appears to be a key mediator of nicotine and cannabinoid-seeking behavior. Systemic SB-334867 administration reduces nicotine self-administration [137], cue-induced reinstatement [138] and orexin-A induced reinstatement of nicotine-seeking [139]. Systemic SB-334867, but not TCS OX2 29, reduces signs of mecamylamine-induced nicotine withdrawal [140] and the operant acquisition, self-administration and break-point for a synthetic cannabinoid agonist, WIN55,212-2 [141]. Additionally, there is emerging evidence for interactions between the orexin system and endogenous cannabinoid signalling [19, 36, 142] and orexin-A expression is downregulated in tetrahydrocannabinol-dependent patients [143]. Collectively, these studies indicate that the OX_1 receptor may have clinical relevance for drug addiction more broadly but the OX_2 receptor is more specifically implicated in mediating the primary reinforcing properties of depressant drugs such as alcohol and opiates.

3.1.5 Orexin and Addiction Neurocircuitry

Regions subjected to orexinergic modulation, like the VTA, nucleus accumbens and the prelimbic cortex may be involved in promoting craving of specific reinforcers and retrieving memories required to perform drug-seeking behaviors. Dopamine release from the VTA [102, 144], expression of *Hcrt2* and orexin fibers [85, 90] converge on the dorsomedial shell of the nucleus accumbens which has been shown to be a hedonic hotspot that mediates "liking", "wanting", promotes food intake [145–147] and encodes specific reinforcers such as water, sucrose, alcohol or cocaine in specific anatomically intermixed neurons [148–150]. Orexin-A in the shell of the nucleus accumbens itself can also increase dopamine concentration, which may be reduced by antagonism of OX_1 receptors or ionotropic glutamate receptors [151]. The closely connected ventral pallidum may also promote "liking" which has been shown after orexin-A microinjection [152]. Meanwhile, the prelimbic cortex has been associated with retrieving memories of action-outcome associations because lesions impair the ability of a rat to selectively avoid devalued actions [153]. This may explain why activation of the prelimbic cortex has been associated with stress, drug-primed [154, 155] and cue-induced reinstatement [156] and context-induced renewal of drug-seeking [157]. In the rat, central administration of orexin-A increases dopamine levels in the prelimbic cortex and activates VTA neurons which project to the prefrontal cortex [144, 158]. Activity in the human analogue of the prelimbic cortex, the dorsal anterior cingulate [159], and several other prefrontal regions is also correlated with craving [160]. Pharmacological antagonism of orexinergic activity may therefore reduce craving of specific reinforcers and the motivational and rewarding processes of mesolimbic circuits.

It has also been suggested that the PV may be involved because it receives significant orexinergic innervation [161], which is activated by nicotine [162] and orexin-A in the PV increases dopamine in the nucleus accumbens [163]. It has been hypothesized that the PV integrates energy balance signals from lateral hypothalamic orexin neurons and then influences the nucleus accumbens to motivate feeding behavior [164]. Orexinergic fibers also appear to be closely associated with PV neurons which are activated during cue-induced reinstatement [120] or project to the nucleus accumbens shell [165]. However, evidence from pharmacological studies is mixed. OX_1 antagonism via intra-PV microinjection of SB-334867 does not reduce cue-induced reinstatement of cocaine-seeking [166] while microinjection of orexin-A precipitates reinstatement of cocaine-seeking [167]. The PV may be a site of OX_2 receptor-mediated effects because both OX-A and OX-B microinjections increase freezing behavior and TCS OX2 29 but not SB-334867 attenuates expression of a conditioned place aversion to naloxone-induced morphine withdrawal [168, 169]. As others have noted [170], the apparently contradictory findings and indirect evidence in the literature suggests that further research is required before drawing strong conclusions about the role of PV orexin signalling in addiction.

3.1.6 Natural and Synthetic Reward Discrimination

Interestingly, the orexin system seems able to differentiate between natural and synthetic rewards under certain conditions. Systemic SB-334867 administration reduces operant responding for both alcohol and 0.5–0.7 % sucrose (w/v) under FR3, yet reduces the break-point for alcohol

but not sucrose in male iP rats [171], though see [115] for conflicting data in female iP rats. SB-334867 reduces reinstatement of alcohol-seeking but not SuperSac (3 % glucose/0.125 % saccharin (w/v)) [172]. It has also been demonstrated that the OX_2 receptor has similar discriminative capacities because central antagonism using TCS OX2 29 or JNJ-10397049 reduces operant responding (FR3) for alcohol, but not 0.7–1 % sucrose (w/v), or 0.1 % saccharin (v/v) respectively [116, 118]. It may also be that the lateral hypothalamic neurons are the source of discrimination because food and water-restricted rats show activation of orexin neurons during context-induced renewal of 4 % alcoholic beer-seeking [173], but not 10 % sucrose-seeking [174]. The VTA also has discriminative capacity because while systemic Almorexant appears to reduce 5 % sucrose self-administration at lower doses than 20 % alcohol, microinjection targeting the VTA selectively reduces 20 % alcohol self-administration [121]. This discriminative capacity has also been demonstrated for stimulants and food, where OX_1 receptor antagonism (SB-334867) reduces fixed-ratio responding and break-point for nicotine self-administration but not food pellets [137]. SB-334867 reduces cocaine self-administration in food-restricted rats, but does not reduce the number of food pellets earned on an FR5 schedule of reinforcement [132]. Neither SB-334867, nor the selective OX_2 receptor antagonist TCS OX2 29 [53] reduce cue-induced reinstatement of food pellet-seeking [138]. Other studies have shown that there is a reduction in sucrose pellet-seeking (FR1) and reinstatement following OX_1 receptor antagonism (SB-334867), but only in food restricted rats [175]. In contrast, the same OX_1 receptor antagonist reduces self-administration of 1 % saccharin pellets and cue-induced reinstatement in both food restricted and sated rats [176]. The orexins' discriminative capacity appears to be based on reward salience, since motivation (break-point) for food is unaffected by SB-334867 but reduced for cocaine and high fat chocolate pellets [177]. Thus it appears that that OX_1 receptors are recruited selectively when levels of consumption or motivation to consume are high [116, 178, 179]. This presents a particular advantage over naloxone, which also reduces responding for a low concentration 0.1 % saccharin (v/v) solution [116]. It should therefore be possible to treat the highly salient cravings that are associated with addiction by antagonism of one or both of the orexin receptors, without affecting motivation for the less salient reinforcers of everyday life such as food and water.

4 Drug Development

If orexin signalling is acting to promote craving then targeting pharmacological antagonists to one or both of the orexin receptors should reduce drug craving and relapse in the clinic. Orexin antagonists currently under development are mainly targeted to the treatment of sleep disorders and so their pharmacology and kinetics may not be optimal for treating addiction but there are some promising signs. Almorexant, a DORA first reported in the literature in 2007 [57], was shown to reduce cocaine and amphetamine CPP yet not morphine CPP [111]. Almorexant also reduces alcohol consumption in rat two-bottle free-choice and progressive ratio paradigms and the mouse drinking-in-the-dark paradigm [115]. Moreover, rats do not learn CPP for Almorexant-paired contexts which suggests orexin antagonism itself is not rewarding [111]. Although humans with experience of non-therapeutic use of depressants do not generally experience euphoria when given Almorexant, they do report liking the drug and make some comparisons with benzodiazepines and opiates [63], suggesting some abuse potential. Due to the focus on DORAs for insomnia and the recency of their development, there are few published studies of DORAs in the drug-literature but the Almorexant studies provide proof-of-principle for use of DORAs to treat addiction.

4.1 Potential Side Effects and Safety

The multiple roles that orexin signalling occupies in the central nervous system suggest many possible side effects from clinical use of orexin antagonists to treat addiction. Animal studies have shown the importance of orexins for feeding [13, 180], motivation [181], sleep/wakefulness [182, 183], male sexual behavior [98] and female proestrus [184] which raises the possibility of side effects such as anorexia, weight loss, anhedonia, and asexuality in addition to observed side effects such as somnolence, fatigue and muscle weakness [58, 60, 63, 79, 80, 185, 186]. Orexin antagonist effects on sleep architecture appear to be more idiosyncratic, with Suvorexant increasing REM sleep [187] while Actelion recently reported a DORA which increases NREM sleep in rats [188]. The hypnotic effects of orexin antagonists raises the possibility of cognitive impairment and some memory impairments have been observed in elderly patients given Almorexant [186]. However, studies on cognitive effects in rats have shown that DORAs have a relatively greater therapeutic window than current treatments for insomnia [189]. Animal studies also suggest possible depressive effects because calorie restriction has antidepressant-like effects in wild type but not orexin-knockout mice [190] and male rats which have experienced social defeat stress and display anhedonic sexual disinterest have reduced orexin-A and orexin-B in the medial prefrontal cortex and hypothalamus and reduced orexin-B in the VTA [191]. However, while there is a

physiological role for the orexin system in depressive behavior this has not been observed in clinical trials which may suggest that doses higher than those used to treat insomnia might be required for such side-effects to manifest. Some side-effects may even be beneficial since activation of orexin neurons is necessary for a panic-prone state in rats [192].

Orexins have also been shown to increase blood pressure in rats [193]. Almorexant administration can reduce blood pressure in spontaneously hypertensive rats, but has no effect on resting blood pressure in normal rats [194] which may provide a beneficial side effect in patients with hypertensive comorbidities. Importantly there appears to be no effect of Almorexant or Merck's DORA-12 on rotarod performance in rats when administered alone or in combination with alcohol [195], indicating that it is unlikely to affect manual coordination or interact with alcohol. Disruption of orexin signalling may also have effects in the periphery since mice have been shown to express *Hcrt1* in several tissues including the vena cava, bladder, stomach and adrenal glands. In contrast *Hcrt2* appears to be more confined to the central nervous system with lower levels of expression in peripheral tissues such as the spleen, liver and bone marrow [196].

The results of clinical trials to date indicate that the safety of DORAs may depend on their selectivity for the orexin receptors and avoidance of off-target effects. Phase I clinical trials for another DORA, SB-649868, did not raise tolerability issues [67] however GlaxoSmithKline discontinued its development following the discovery of an unspecified safety issue in rats [68]. Published reports of the binding affinity and potency of SB-649868 (summarized in Table 3) show that it has high affinity and is a potent antagonist in the nM range [66, 197]. The most advanced DORA, Suvorexant [78], is likely to receive FDA approval once Merck makes a lower 10 mg dose available [76]. Although it is slightly less potent than SB-649868 or Almorexant, it is >10,000 times more selective for the orexin receptors than 170 other receptors, including GPCRs, ion channels and enzymes [77]. SB-649868 is only >1,000 times more selective for orexin receptors [66] and Almorexant inhibits several targets with an IC_{50} in the μM range, including CB_1 and CB_2 receptors and L-type Ca^{2+} channels [57] although its discontinuation was for other reasons. Filorexant was run through the same selectivity screen as Suvorexant and is reportedly highly selective for orexin receptors [71] although it appears clinical trials have been terminated for unstated reasons [75]. If off-target effects were behind the discontinuation of SB-649868 it may have implications for other DORAs and there may be a safety advantage with SORAs.

4.2 Target Selection: SORA or DORA?

It is not yet clear whether DORAs are the most appropriate type of orexin receptor antagonist for the treatment of addiction. A SORA may even be more appropriate given the concentration of preclinical evidence around the OX_1 receptor. SB-334867 has been relied upon in many addiction studies even though it can have off-target effects on the OX_2 receptor [49] and has recently been shown to hydrolyse to an inactive state when it is kept as a hydrochloride salt [198]. This explains discrepancies in the doses used across different studies. For example, the first study on OX_1 receptors and alcohol-seeking in rats used a 20 mg/kg dose of SB-334867 which abolished cue-induced reinstatement and attenuated self-administration, while a later study from the same lab used 5 mg/kg to attenuate self-administration [112, 171]. However, studies on the OX_2 receptor have failed to show effects on cue-induced reinstatement of alcohol-seeking [118], cocaine-seeking and cocaine self-administration [124]. Although accumbal OX_2 receptors have been shown to be upregulated following chronic cocaine administration (20 mg/kg, i.p.) [199] this may have more to do with mediating the rewarding properties of addictive drugs than the appetitive or drug-seeking phase. This is supported by findings that OX_2 receptor antagonism using JNJ-10397049 reduces CPP for alcohol [116], while OX_1 receptor antagonism does not [200]. A more recent study has also found no change in OX_2 receptor levels following withdrawal from cocaine self-administration [201].

The choice between a DORA and a SORA is likely to depend on a range of practical and clinical considerations, such as development costs, treatment aims, patient profile including drug(s) of abuse and effect size. It makes little theoretical sense using a DORA to treat a patient with cocaine addiction, given that systemic administration of TCS OX2 29 has not been shown to affect cocaine self-administration or cue-induced reinstatement while SB-334867 reduces reinstatement [124]. A patient with alcoholism, on the other hand, may benefit from a DORA because three separate preclinical studies, each from a different lab using a different OX_2 receptor antagonist, have all implicated the OX_2 receptor in alcohol consumption [115, 116, 118]. However, pharmaceutical companies may have insufficient incentive to develop an OX_1 receptor antagonist specifically for addictions where only OX_1 receptors are implicated when the DORAs for insomnia are on the verge of FDA and PMDA approval. Even if a SORA might be ideally suited for the treatment of a particular patient, trialling a DORA like Suvorexant may be a better option in the short to medium term because its safety at lower doses has already been established. The treatment aim is also an important consideration because drug-

Table 3 Published affinities and potencies of clinically tested dual orexin receptor antagonists

Ligand	Measure[a]	OX_1	OX_2	References
Almorexant (ACT-078573)	pK_i—radioligand-displacement binding assay	8.6	9.7	[71]
	IC_{50}—Orexin-A induced Ca^{2+} fluorescence	13 nM	8 nM	[57]
	pK_b—Orexin-A induced IP1 accumulation	8.4	9.0	[197]
	pK_b—Orexin-A induced Ca^{2+} fluorescence	6.9	6.9	[71]
	pK_b—Orexin-A induced Ca^{2+} fluorescence[b]	7.8	8.3–9.1	[212]
SB-649868	pK_i—Radioligand-displacement binding assay	9.5	9.4	[66]
	pK_b—Orexin-A induced IP1 accumulation	9.7	9.6	[197]
	pK_b—Orexin-A induced Ca^{2+} fluorescence[b]	9.0–9.4	9.5–9.8	[212]
Filorexant (MK-6096)	pK_i—Radioligand-displacement binding assay	8.6	9.5	[71]
	pK_b—Orexin-A induced Ca^{2+} fluorescence	9.0	8.0	[71]
	pK_b—Orexin-A induced IP1 accumulation	9.1	9.8	[197]
	pK_b—Orexin-A induced Ca^{2+} fluorescence[b]	9.1–8.9	9.4–9.8	[212]
Suvorexant (MK-4305)	pK_i—Radioligand-displacement binding assay	9.3	9.5	[77]
	IC_{50}—Orexin-A induced Ca^{2+} fluorescence	50 nM	56 nM	[77]
	pK_b—Orexin-A induced Ca^{2+} fluorescence[b]	8.4–8.7	9.0–9.2	[212]

[a] pK_i refers to equilibrium dissociation constants from competitive radioligand binding studies and pK_b refers to results from functional assays [213]. All values are from studies on human orexin receptors

[b] The first value in the range given is the reported value after 30 min and the second value is after 4 h

seeking and taking are multifaceted and the two orexin receptors have been shown to mediate drug effects through distinct neuroanatomical and behavioral patterns in rats [202]. An OX_1 receptor antagonist may again be best suited if the sole treatment aim is to reduce relapse. However, clinicians are often interested in both promoting abstinence and reducing the amount of drug used if a patient does relapse [203, 204]. The treatment aim will also influence what clinicians are likely to consider to be relevant for their patients. A survey of 50 substance abuse treatment providers has shown that a 50 % increase in the abstinence rate (from 25 to 38 %) would be considered clinically significant, but a new therapy would have to halve the number of drinking days and drinks per drinking day to interest providers in adopting it [205]. A relapse risk difference of at least 10 % also appears in line with patterns of FDA approval for pharmacotherapies for psychiatric disorders, such as bipolar disorder or depression, where the number needed to treat for one person to benefit (NNT)[1] for approved drugs are typically less than 10 [206–208] and acamprosate was thought that have an NNT of 9.09 [209]. Certainly, individual studies in preclinical animal models are able to achieve such effect sizes but translational researchers might be more willing to bet on a DORA to deliver large enough effects [183] in the clinic where the patients have not been bred under controlled conditions.

5 Conclusions

The orexin/hypocretin system has an established role in reward-seeking and drug addiction and is a candidate target for future pharmacotherapies for addiction. It is particularly inviting as a mediator of salient rewards and its distribution throughout the brain makes it well placed to mediate both craving and the rewarding and reinforcing properties of drugs, as well as drug-seeking behavior. However, it has highly complex pharmacology that has the potential to interact directly with other systems, such as the endocannabinoids because orexin receptors may complex with cannabinoid receptors or activate them via paracrine signalling [36, 142]. The failure of two DORAs in clinical trials, SB-649868 and Almorexant, raises safety concerns and the FDA response to Merck's Suvorexant states that high doses have not been shown to be safe [76] which suggests the class has a low therapeutic index. Several questions remain regarding the clinical use of orexin receptor antagonists for addiction, such as whether to target OX_1 alone or to inhibit both receptors as has been the approach for insomnia. While it is likely that treating addiction may become an 'off-label' or additional use for Suvorexant, an orexin antagonist which has been custom made to modify the optimal receptor(s) and signalling mechanisms to best treat addiction may be somewhat further in the future.

[1] The number needed to treat (NNT) is calculated as 1/risk difference. An increase in abstinence rate from 25 to 38 % gives a risk difference of 13 % and an NNT of 8.

Acknowledgements RMB and SYK are supported by the National Health and Medical Research Council of Australia. SYK is supported by an Australian Postgraduate Award; RMB is supported by a Peter

Doherty Fellowship. The authors declare that they have no conflicts of interest.

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