CLINICAL INVESTIGATION

Hypocretin receptor antagonists for insomnia: rationale and clinical data

Clin. Invest. (2012) 2(6), 623–637

Loss of hypocretin neurons leads to sleep attacks and cataplexy in a rare sleep disorder known as narcolepsy. The hypocretin system stabilizes states of wakefulness and sleep through complex neural pathways. Insomnia, on the other hand, is a common sleep disorder characterized as difficulty initiating or maintaining sleep. Current treatment options for insomnia are limited to cognitive-behavioral therapy and pharmacological agents such as non-benzodiazepine receptor agonists. The discovery of hypocretin and deficiency of these peptides in narcolepsy spurred the development of pharmacological agents that antagonize hypocretin receptors for the treatment of sleep disorders such as insomnia. Preclinical and limited clinical data on hypocretin antagonists have shown favorable outcomes in the treatment of insomnia but concerns of theoretical adverse effects still remain.

Keywords: almorexant • cataplexy • hypocretin • insomnia • MK-4305 • MK-6096K • narcolepsy • orexin • SB-649868 • suvorexant

Chad Ruoff* & Christian Guilleminault
Stanford University Sleep Medicine, Stanford University School of Medicine, Stanford, CA, USA
*Author for correspondence:
Tel.: +1 650 721 7564
Fax: +1 650 721 3465
E-mail: cruoff@stanford.edu

Loss of hypocretin-producing neurons in narcolepsy with cataplexy

A review of the clinical presentation and associated findings in narcolepsy with hypocretin deficiency highlights potential, therapeutic benefits of antagonizing the hypocretin system with regards to the sleep–wake cycle, but also identifies potential, untoward effects of antagonizing the hypocretin system. This provides a list of theoretical side effects of hypocretin antagonists that require careful evaluation in the development of this new compound class. Narcolepsy with cataplexy in humans, is associated with the destruction of hypocretin-producing neurons, which produce hypocretin and other colocalized peptides such as glutamate, dynorphin and NARP [1]. Neuropathological studies have shown up to a 95% reduction in hypocretin-producing cells [2,3]. Hypocretin is undetectable in the cerebrospinal fluid (CSF) in most individuals with narcolepsy with cataplexy [4,5].

The cardinal features of narcolepsy with hypocretin deficiency are excessive daytime somnolence and cataplexy, a transient loss of postural muscle tone during wakefulness often triggered by positive, emotional stimuli such as laughter. Cataplexy manifests most often as a transient buckling of the knees, head dropping, or sagging of the jaw but can escalate to frank paralysis of all antigravity muscles for several minutes. The excessive daytime sleepiness in narcoleptic patients may occur in waves of irresistible sleep attacks, transitioning rapidly from wakefulness to non-rapid eye movement (NREM) and/or rapid eye movement (REM) sleep, typically during times of monotonous, sedentary activities, but may also occur while conversing, eating or riding a bicycle. Short naps are usually refreshing for narcoleptics. Although these sleep attacks lead to fragmented sleep–wake patterns, the total sleep time (TST) across the 24-h cycle is similar to normal individuals [6].

FUTURE SCIENCE part of fsg

Other REM-related sleep phenomena, which can occur in normal individuals especially if sleep deprived, include hallucinations and sleep paralysis. Hallucinations tend to occur during REM sleep, a state of sleep associated with dreaming, during which normal brainstem function inhibits motor activity. Sleep paralysis and hallucinations often occur concurrently at the transition from wakefulness to sleep, or *vice versa*, and may persist for up to several minutes. The paralysis prevents the individual from making any voluntary body movements – so much as even lifting a finger. In addition to cataplexy, the intrusion of REM sleep into wakefulness may manifest as sleep paralysis and/or hallucinations [6].

Other reported associated findings in narcolepsy include abnormalities in metabolic, hypothalamic–pituitary axis, autonomic and neuropsychiatric functions as well as comorbid sleep disorders. Narcoleptic patients tend to have an increased body-mass index and an increased risk of developing insulin-resistant diabetes mellitus. Precocious puberty has also been reported in early-onset cases [6]. Autonomic dysfunctions such as lower body temperature and altered cardiovascular parameters, including heart rate variables and blood pressure, have been reported [7–9]. Learning problems and impaired concentration difficulties as well as depression have also been reported [6]. They also demonstrate motor dyscontrol during REM sleep and an increased frequency of periodic limb movement syndrome [10]. Periodic limb movement syndrome is characterized as repetitive, involuntary limb movements during sleep, typically described as extension of the big toe with flexion on the knee. Narcoleptics are also at increased risk of upper-airway resistance syndrome and obstructive sleep apnea (OSA) [11].

Characterization of hypocretin neuropeptides & receptors in humans

The hypocretin system is a collection of 50,000–80,000 hypocretin-producing neurons located in the lateral hypothalamus with projections throughout the CNS [12]. Co-discovered over a decade ago, the hypocretin system comprises of two hypocretin neuropeptides, HCRT-1 and -2 (also known as orexin A and B, respectively). One group named them hypocretins since they are located within the hypothalamus and are structurally similar to secretin, a digestive peptide, while the other group named them orexins after the Greek word 'orexis', which means appetite, since intracerebroventricular (ICV) injection stimulated food intake [13,14]. Preprohypocretin, a precursor polypeptide composed of 130 residues, undergoes proteolytic cleaving to produce HCRT-1 and -2 with 33 and 28 amino acids, respectively [14]. HCRT-1 is highly lipophilic, crosses the blood–brain barrier via simple diffusion and is stable in the CSF. On the other hand, HCRT-2 is a linear peptide that is not stable in the CSF and has a short biological half-life due to rapid metabolism and clearance from the body [15–17]. The hypocretin neuropeptides bind to two G-protein coupled receptors (GPCRs), the HCRT-1 and -2 receptors. HCRT-1 receptor is a selective receptor with a very high affinity for HCRT-1, while HCRT-2 receptor is a nonselective receptor with equal affinity for both HCRT-1 and -2. Although the HCRT-1 receptor share some similarities to other GPCRs such as the TRH receptor, cholecystokinin type-A receptor and Y2 NPY receptor, neither HCRT-1 or -2 have significant affinity to these other GPCRs [14].

Physiologic implications of the hypocretin system

Hypocretin & the sleep–wake cycle

Prior to the discovery of hypocretin deficiency in human narcoleptic patients, hypocretin knockout mice and familial canine narcolepsy, which is due to an autosomal recessive mutation in the HCRT-2 receptor gene, were shown to demonstrate very similar phenotypes to human narcolepsy. It has also been demonstrated that hypocretin knockout mice demonstrate much more severe cataplexy than the HCRT-2 receptor knockout mice and the HCRT-1 receptor knockout mice exhibit no signs of cataplexy [6]. Interestingly, asymptomatic, heterozygous canines demonstrate cataplexy when given cholinergic medications and medications that decrease monoamine transmission (e.g., prazosin) without altering the hypocretin levels in the CSF [18,19]. In contrast, normal dogs do not demonstrate cataplexy when administered these same medications but do demonstrate changes in hypocretin levels in the CSF [18].

ICV injection of hypocretin in rats and mice during the rest period has been shown to promote wakefulness and decrease REM and NREM sleep [20]. Physiologic fluctuations in hypocretin levels have been reported, with levels increasing to a peak toward the end of the active period – possibly to counter the growing homeostatic pressure – before levels decline with the onset of sleep [21–26]. In HCRT-1 and -2 knockout mice, the effects of ICV injection on wakefulness and NREM sleep are reduced, when compared with wild-type mice, with a less robust response noted in the HCRT-2 receptor knockout mice. Suppression of REM sleep by hypocretin injection was similarly attenuated in both knockout mice [27]. In a different animal model, a transgenic hypocretin/ataxin-3 mouse model, which leads to the postnatal death of hypocretin neurons, ICV HCRT-1 augments wakefulness and decreases both frequency and duration of cataplexy [28]. Other studies are investigating

the downstream effects of the hypocretin system. For example, it has been shown that ICV injection of hypocretin in histamine-1 (H_1) receptor knockout mice and in wild-type rats in combination with a selective hypocretin antagonist (pyrilamine) in wild-type rats blocks the wake-promoting effects of hypocretin; however, H_1 receptor and H_1/HCRT-1 receptor knockout mice demonstrate normal sleep–wake states [29–31]. Lastly, optogenetic studies show that repeated stimulation of HCRT neurons increases the probability of an awakening during sleep and this effect was not observed in HCRT knockout mice [32].

In summary, it is suggested that the profound dysregulation in REM sleep in narcolepsy is dependent upon both hypocretin receptors but that activation of the HCRT-2 receptor promotes wakefulness and suppresses NREM sleep with higher efficacy than stimulation of the HCRT-1 receptor [27,33]. Further investigations are required in order to better elucidate the downstream effects of the hypocretin system on the sleep–wake cycle. For a complete review of the neurobiology of sleep refer to reference [34].

■ Hypocretin, feeding behavior & energy expenditure

There remains significant controversy over whether the hypocretin system, independent of its effects on arousal and locomotion, influences feeding behavior. Several studies have shown that ICV administration of hypocretin induces feeding behavior and hypocretin mRNA expression increases during fasting [20]. Individuals with narcolepsy with hypocretin deficiency tend to have an increased body-mass index in spite of decreased caloric intake and an increased risk of developing insulin resistant diabetes mellitus [35–37]. The transgenic hypocretin/ataxin-3 model mice are more obese than the preprohypocretin knockout mice, but these transgenic mice fail to respond to fasting with increased wakefulness and activity [38,39]. These metabolic effects might be, in part, due to colocalized factors in HCRT neurons such as dynorphin, NARP and glutamate, which are absent in the transgenic hypocretin/ataxin-3 model [38]. On the contrary, fasting in dogs and rodents does not increase hypocretin levels in the CSF. Furthermore, chronic ICV administration of hypocretin in rats for 7 days did not modify overall food intake or cause obesity but did lead to a differential response in food intake depending upon the sleep cycle. During the resting phase, animals remained awake and, likely as a consequence, showed an increase in food consumption [40]. The presence of receptors for leptin, which is a satiety hormone, on hypocretin neurons, however, does suggest that this system is able to directly respond to metabolic signals. Activity of isolated hypocretin neurons is inhibited by glucose and leptin and stimulated by ghrelin, which is an appetite stimulating hormone [20,40].

■ Hypocretin, the autonomic nervous system & ventilation

The hypocretin system has also been implicated in the control of the autonomic system and the cardiopulmonary system, which is no surprise given the widespread projections of the hypocretin system throughout the hypothalamus and the brainstem, including projections to the nucleus tractus solitaries and the pre-Botzinger region [41,42]. ICV injection of hypocretin increases blood pressure, heart rate, sympathetic tone, plasma norepinephrine and epinephrine and respiratory rate and tidal volume [43,44]. Transgenic hypocretin/ataxin-3 have lower blood pressures [45]. Hypocretin neurons, *in vitro* and *in vivo*, increase c-FOX expression in response to increased carbon dioxide and hypocretin knockout mice demonstrate attenuated central chemoreception during wakefulness [46–48].

■ Hypocretin, reward, addiction & pain

There is a growing body of evidence that the hypocretin system is involved in reward and addiction pathways. It has been observed for decades that patients with narcolepsy with cataplexy treated with alerting agents rarely have problems with drug abuse and addiction. In addition, hypocretin knockout mice exhibit abnormal baseline dopamine signaling with a dampened response to cocaine and morphine [49,50]. ICV injection of hypocretin leads to the reinstatement of previously extinguished cocaine-seeking behaviors in rats [51].

The presence of HCRT-1 and HCRT-1 receptors in the dorsal root ganglion cells and white/grey matter of the spinal cord suggests that the hypocretin system might also be involved in pain pathways. Furthermore, intravenous or ICV hypocretin has been shown to be an effective antinociceptive and antihyperalgesic agent in mice and rats [52].

■ The pituitary–hypothalamic–adrenal axis & hypocretin

The presence of peripheral hypocretin receptors suggests that this system may also be implicated in the periphery. There are reports of hypocretin neuropeptides in the plasma but it should be emphasized that detection of hypocretin in the plasma is currently very difficult [53]. HCRT-1 and -2 receptors are expressed in the male reproductive system including the testis, epididymis, seminal vesicles and penis [54]. HCRT-1 and

-2 neuropeptides and receptors have also been identified in the anterior pituitary [55]. Preprohypocretin mRNA is found in the stomach, kidney, adrenal gland, pancreas, colon, placenta, colon and white adipose tissue [56,57]. ICV hypocretin has been shown to decrease plasma growth hormone and prolactin and increase corticosterone and ACTH levels [58,59]. ICV administration of HCRT-1 has also been shown to inhibit pulsatile luteinizing hormone when food is withheld [60].

In summary, the primary role of the hypocretin system appears to be in the regulation of arousal, but it may also serve as the interface between multiple other physiologic functions, including metabolism, reward and addiction, hypothalamic–pituitary–adrenal axis, ventilation and autonomic nervous system control.

Hypocretin antagonists in the treatment of insomnia: preclinical & clinical data

Sleep research over the last decade has led to a new class of pharmacological agents that antagonize the hypocretin system. The effects of hypocretin deficiency upon the sleep–wake cycle in narcolepsy (e.g., sleep attacks, excessive sleepiness and short, refreshing naps) suggest that the administration of hypocretin antagonists to individuals with insomnia may help to promote sleep. Insomnia is a disorder characterized by difficulty falling asleep and/or maintaining sleep associated with a poor quality of sleep or nonrestorative sleep [61]. It leads to functional daytime impairment in about 10% of adults [62]. Available treatment options are limited to cognitive-behavioral therapy and pharmaceutical agents. The most prescribed and investigated therapy for insomnia is benzodiazepine and non-benzodiazepine receptor agonists. Other prescription and over the counter medications used in the treatment of insomnia include alcohol, acetyl-salicylate, antidepressants, antipsychotics, histamine receptor antagonists, melatonin receptor agonists and herbal remedies. These medications have limited efficacy and adverse effects including metabolic and cognitive effects, rebound insomnia, abuse potential, dependence, tolerance and impaired motor skills.

This has led to numerous patents for dual hypocretin receptor, selective HCRT-1 receptor and selective HCRT-2 receptor antagonists (Table 1) [63]. Preclinical and limited published clinical data have shown that this new compound class does, in fact, promote sleep in animals, healthy normal patients and in patients with chronic primary insomnia. This new compound class has been shown to significantly affect REM sleep more than slow wave sleep (SWS) when compared with zolpidem. Preclinical data also suggest that these compounds do not lead to dependence but rather may serve as a treatment for drug withdrawal and drug-seeking behavior [64]. Although no published data specifically address the risk of rebound insomnia with these medications, one might speculate that the risk of rebound insomnia with this compound class is less than other medications such as zolpidem. In addition, if true, cognitive-behavioral therapy with or without a hypocretin antagonist may dramatically improve acute and long-term management of insomnia [65]. However, the implicated functions of the hypocretin system as discussed raise multiple theoretical concerns for the development of this new compound.

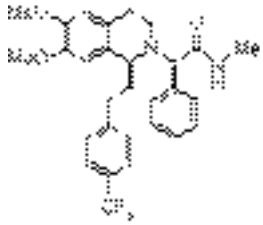
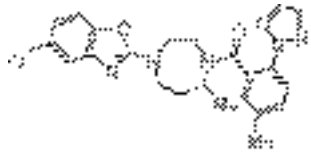
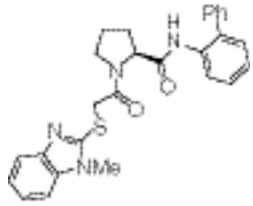
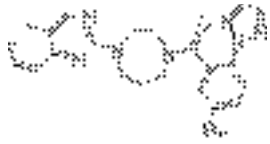
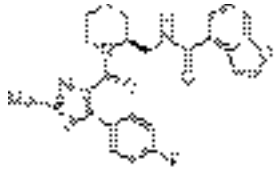
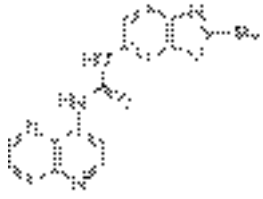
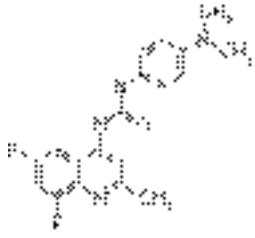
Only two dual hypocretin receptor antagonists, almorexant and suvorexant, have reached Phase III trials. There are other dual hypocretin and selective hypocretin antagonists with favorable preclinical data, including two other dual hypocretin receptor antagonists, SB-649868, which has completed a Phase II trial, and MK-6096, which has completed a Phase I trial.

Unfortunately, in early 2011, Actelion and GlaxoSmithKline stopped further development of almorexant due its clinical and tolerability profile and have not since disclosed further details [101]. Suvorexant does not have any active clinical trials but recently completed several trials and plans on filing a new drug application with the US FDA in 2012 [102]. The withdrawal of almorexant development, despite a Phase III trial demonstrating superiority over placebo on both objective and subjective wake after sleep in adult patients with chronic primary insomnia, should serve as a reminder of the inherent complexity of the hypocretin system [101]. Therefore, a review of the available clinical data, unpublished clinical trials and pertinent preclinical data on hypocretin antagonists pertaining to sleep–wake regulation and other implications of these compounds, is helpful in guiding future development.

Almorexant

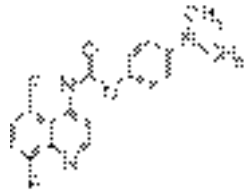
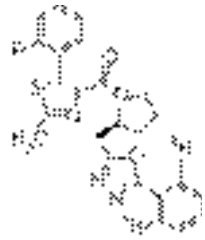
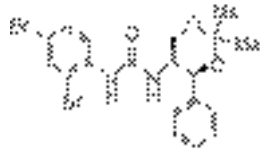
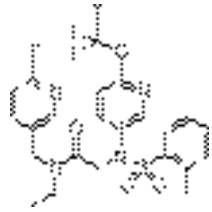
Almorexant (ACT-078573; [2R]-2-[(1S)-6,7-dimethoxy-1-(2-[4-(trifluoromethyl)phenyl]ethyl)-3,4-dihydroisoquinolin-2(1H)-yl]-*N*-methyl-2-phenylacetamide) was identified by Actelion in 2007. It is an orally active tetrahydroisoquinoline derivative that is rapidly absorbed in the fasting state and readily crosses the blood–brain barrier, where it acts as a selective and reversible, dual hypocretin receptor antagonist with little affinity to other receptors, including those for histamine, opiates and GABA [66,67]. It acts as a competitive antagonist of HCRT-1 but a partially noncompetitive antagonist of HCRT-2. In hamster ovary cells that overexpress human HCRT-1 and -2 receptors, it has a half maximal inhibitory concentration of 13 ± 1 and 8 ± 1 nM, respectively [68]. In 2008, Actelion joined forces with GlaxoSmithKline to further develop and market the

Table 1. Hypocretin antagonists studied in preclinical and clinical trials to date.

Compound	Selectivity	Structure	HCRT-1 affinity	HCRT-2 affinity	Ref.
Almorexant	Dual		IC_{50} = 13 nM	IC_{50} = 8 nM	[66]
Suvorexant	Dual		K_i = 0.55 nM IC_{50} = 50 nM	K_i = 0.35 nM IC_{50} = 56 nM	[84]
MK-6096	Dual		K_i = 2.5 nM IC_{50} = 11 nM	K_i = 0.3 nM IC_{50} = 11 nM	[85]
Merck DORA-1	Dual		K_i = 3 nM IC_{50} = 17 nM	K_i = 0.2 nM IC_{50} = 4 nM	[86,87]
Merck DORA-5	Dual		K_i = 1.2 nM IC_{50} = 29 nM	K_i = 0.6 nM IC_{50} = 27 nM	[88]
SB-649868	Dual		pK_i = 9.8	pK_i = 9.3	[89]
SB-334867	HCRT-1		K_b = 27.8 nM	K_b = 1704 nM	[90]
SB-408124	HCRT-1		K_b = 21.7 nM	K_b = 1405 nM	[90]

Dual: Dual hypocretin antagonist.

Table 1. Hypocretin antagonists studied in preclinical and clinical trials to date (cont.).

Compound	Selectivity	Structure	HCRT-1 affinity	HCRT-2 affinity	Ref.
SB-410220	HCRT-1		K_b = 8.8 nM	K_b = 503 nM	[90]
SB-674042	HCRT-1		K_b = 1.1 nM	K_b = 129 nM	[90]
JNJ-10397049	HCRT-2		pK_i = 5.5	pK_i = 8.3 pK_b = 7.9	[87,76]
EMPA	HCRT-2		K_i > 900 nM	K_i = 1 nM	[91]

Dual: Dual hypocretin antagonist.

drug. Almorexant has several Phase I and II trials and one Phase III trial.

In a Phase I trial in 2007, Brisbare-Roch *et al.* reported that almorexant induced somnolence in a dose-dependent manner in humans as well as rats and dogs. Healthy, male human subjects (n = 70; aged 18–37 years) were randomized in a double-blinded fashion to receive a single dose of almorexant (n = 42 with n = 6 per dose group: 1, 5, 25, 100, 200, 400 or 1000 mg), 10 mg zolpidem (n = 14), or placebo (n = 14) at approximately 10:00. Almorexant doses ≥200 mg led to a significantly shortened latency to stage 2 sleep, improved sleep efficiency, increased total sleep time and increased NREM duration; however, doses of at least 400 mg were required to significantly decrease sleep latency. These findings disappeared 6.5 h after administration of all doses <1000 mg when compared with placebo [66]. It should be emphasized that this study was performed in healthy male subjects without chronic primary insomnia. It appears that EEG monitoring was conducted in 1-min intervals just prior to and at various time points after drug administration for 12 h. In addition, two 25-min EEG recordings were performed 90 min and 6.5 h after dosing. Although the authors of this study reference this as a variant of the multiple sleep latency test (MSLT), researchers are encouraged to investigate the probability for sleep-onset REM periods with the more conventional MSLT on all future studies with hypocretin antagonists [69]. Power spectral analysis of the 25-min recording collected 90 min after drug ingestion revealed an increase in the delta and theta power with almorexant but not with zolpidem [66]. No sleep-onset REM periods were reported based upon the two 25-min EEG recordings performed at 90 min and at 6.5 h after dosing. These recordings were analyzed automatically based upon Rechschaffen and Kales criteria [70]. At this point, all future studies should continuously monitor EEG to more accurately report the effects of hypocretin antagonists on sleep architecture.

In addition to no sleep-onset REM periods when evaluated at 90 min and 6.5 h after dosing, Actelion has not disclosed any reports to suggest that almorexant causes cataplexy, sleep attacks, sleep paralysis or sleep hallucinations [66,68]. Although theoretical concerns for

REM-related phenomenon remain based upon human narcolepsy with hypocretin deficiency and past animal models, Brisbare-Roch *et al.* specifically report that no cataplexy was observed [66]. However, the authors do emphasize that no specific attempts were made to trigger cataplexy and subjects were confined to the study center for 48 h after study drug administration. Merck has completed a Phase III trial on suvorexant conducted over 12 months to evaluate the proportion of patients that experience narcolepsy-like events, such as cataplexy, as one of the primary outcome measures [103]. Interestingly, it appears that Merck still intends on filing a new drug application with the FDA this year [102]. In future studies, researchers should attempt to trigger cataplexy by encouraging positive emotional activity such as laughter in subjects. Certain medications such as prazosin have been reported to exacerbate cataplexy in narcoleptic patients; therefore, individuals being administered hypocretin antagonists with other medications such as prazosin should be closely monitored and potentially evaluated in future studies [19,71].

Subjective alertness was significantly diminished when doses of 400 mg or more were given to subjects [66]. One subject in the 400-mg almorexant group and three subjects in the 1000-mg group reported an increase in dreaming on a narcoleptic questionnaire. One subject at 200-mg dose of almorexant, two subjects at 1000-mg dose of almorexant and one subject in the zolpidem group reported short-lasting muscle relaxation/weakness [68]. No further details were provided to further characterize this short-lasting muscle relaxation/weakness.

Actelion specifically reported no significant effect of almorexant on the following venous blood tests taken before and at regular time points up to 36 h after dosing: ACTH, cortisol, CRF, ghrelin, HGH, leptin, LHRH, orexin A, pancreatic polypeptide, prolactin, testosterone and TSH [68]. This is very significant given past studies, as previously discussed, in rodents documenting changes in several of these values after ICV injection of hypocretin [58–60]. The authors reported no apparent drug-related effects on blood pressure, which was measured in the standing and supine position, heart rate, electrocardiogram and physical examination [68]. These are important findings in light of the prior studies discussed showing a relationship between the hypocretin system and blood pressure control [43,45].

Almorexant, at doses of ≤100 mg, demonstrated a side-effect profile similar to placebo [66,68]. Adverse events associated with almorexant of ≥200 mg when compared with zolpidem included somnolence (33 vs 36%), dizziness (7 vs 43%), diplopia (5 vs 29%), disturbance in attention (10 vs 14%) and fatigue (12 vs 7%). Only subjects treated with zolpidem reported abnormal coordination (n = 4) and 'feeling drunk' (n = 2).

Actelion also completed a Phase II multicenter, double-blinded, randomized, placebo-controlled, five-period, five-treatment crossover, dose-finding study to evaluate the efficacy and safety of almorexant in elderly subjects with chronic primary insomnia with a primary outcome of mean wake time after sleep onset, but there are no publicly available data from this trial [104].

In a Phase III, multicenter, double-blinded, randomized, placebo-controlled, active reference (zolpidem), parallel-group polysomnography (PSG)-based trial (RESTORA 1), almorexant (100 and 200 mg) was evaluated for safety and efficacy across 16 days in 709 adult patients with chronic primary insomnia [72]. The study showed superiority over placebo on both objective and subjective wake after sleep onset (WASO; $p < 0.001$) [105]. Several secondary end points were also statistically significant but no further details are available. This is the first landmark study to demonstrate hypocretin antagonists as effective treatment for patients with chronic primary insomnia. It should be emphasized that superiority was demonstrated only for WASO, not for sleep latency, while zolpidem, a benzodiazepine agonist, is primarily used for sleep-onset insomnia [73]. Recall that dosages of at least 400 mg were required to significantly decrease sleep latency in healthy human subjects [66]. Importantly, the study showed that it significantly improved subjective WASO. This is a key finding for this new compound class as some might argue, including patients with chronic insomnia, that a sleeping pill that objectively improves sleep without a subjective benefit is of little value.

In spite of Actelion and GalaxoSmithKlein abandoning further development of almorexant in early 2011, there is an active, Phase III, randomized, double-blinded, placebo-controlled, parallel-assignment trial comparing the effects of almorexant (100 or 200 mg) and zolpidem (10 mg; n = 216) on neurocognitive performance [106]. There are no preliminary data reported from this clinical trial.

■ Relevant preclinical data for almorexant & other hypocretin antagonists

In reference to this ongoing clinical trial with almorexant, preclinical data investigating the effects of almorexant on learning in rats demonstrated no impairment in spatial learning [74]. In addition, a preclinical trial in rats found that the combination of ethanol and zolpidem, but not ethanol and almorexant, was associated with a dose-dependent decrement in forced-motor performance, which is a surrogate marker for sedation and myorelaxation [67].

The animal data in Brisbare-Roch *et al.* noted male beagle dogs as having an increased frequency of distal muscle movements while resting in a relaxed sleep state starting 2 h after being dosed with almorexant and persisted for the remaining 4 h of the video experiment [66]. This suggests possible muscle dysregulation but the authors emphasized that frequent limb twitching has been reported in dogs during REM sleep. In CAG/hypocretin transgenic mice, which overexpress preprohypocretin by a β-actin/cytomegalovirus hybrid promoter, exhibit sporadic increases in postural muscle tone with the onset of REM sleep along with striking increases in phasic twitches [75]. In addition, patients with narcolepsy exhibit an increased frequency of periodic limb movements [10]. The authors do not explicitly mention whether or not electromyelographic data or if video was acquired and reviewed in human subjects; however, one could assume electromyelographic activity was acquired since Rechschaffen and Kales criteria was used – albeit in an automated fashion – to score sleep stages [70]. Future studies should more closely evaluate and characterize muscle activity during wakefulness and sleep in human subjects before and after ingestion of hypocretin antagonists.

Preclinical data show that hypocretin antagonists promote sleep but suggest that selective HCRT-2 receptor antagonists may be more effective and results may vary according to time of day of administration. Almorexant and a selective HCRT-2 receptor antagonist JNJ-10397049, but not the HCRT-1 receptor antagonist SB-408124, cause a decrease in extracellular histamine within the lateral hypothalamus that parallels the sleep promoting effects of these two compounds. Administration of JNJ-10397049 in either phase of the light–dark cycle decreased latency to NREM and increased NREM and REM sleep time [76]. Selective HCRT-1 receptor antagonists, SB-408124 and SB-334867, had no effect on latency to and duration of NREM and REM sleep regardless of time administered [76,77]. Almorexant administered during both phases of the light–dark cycle increased the duration of NREM and REM sleep but only significantly decreased latency to NREM and REM sleep during the light phase. Lastly, coadministration of a selective HCRT-1 receptor antagonist SB-408124 with the selective HCRT-2 receptor antagonist JNJ-10397049 counteracted the HCRT-2 receptor antagonist induced decrease in NREM latency and increase in NREM duration, but led to a further reduction in REM latency and a nonsignificant increase in REM duration [76]. The further decrease in REM latency could increase the likelihood of inducing REM-related phenomenon such as cataplexy as seen in narcolepsy. Interestingly, recall that hypocretin knockout mice have more severe cataplexy than HCRT-2 receptor knockout mice [72]. Most importantly, these preclinical data suggests that the sleep-promoting effects of a selective HCRT-2 receptor antagonist are disrupted by inhibition of the HCRT-1 receptor and time of day administered influences their effects upon sleep architecture.

In the Brisbare-Roch *et al.* study, the human subjects were dosed at only 10:00 but the rats were dosed at two different times: 17:00 and 6:00 [66,68]. Interestingly, the rats that were dosed at 17:00 demonstrated decreased wake time but rats dosed at 6:00 (i.e., the beginning of the rats normal sleep cycle), did not demonstrate a significant reduction in activity or alertness [66]. As discussed previously, hypocretin levels fluctuate in a slow diurnal pattern and these variations might help explain these observations [22–25]. Alternatively, there might have not been enough time to demonstrate a sleep effect in the normal sleep phase of the rat. Future clinical trials are needed to investigate the response to dual and selective hypocretin antagonists dosed at different times across the 24-day period.

Preclinical data show that hypocretin antagonists may affect drug addiction and abuse behaviors as well as feeding behaviors. Almorexant and a HCRT-1 receptor antagonist SB-408124, but not JNJ-10397049, increased extracellular dopamine within the prefrontal cortex [76]. Almorexant and a HCRT-1 receptor antagonist SB-334867 significantly decreased nicotine self administration [78]. SB-334867 was also shown to decrease self administration of cocaine, block cocaine-mediated behavioral sensitization and decrease excitatory currents in dopaminergic neurons of the ventral tegmental area [49]. Most studies investigating the role of hypocretin antagonists on drug abuse and addiction have focused primarily upon SB-334867. It does appear that the HCRT-1 receptor rather HCRT-2 receptor signaling is integral to the reinstatement of cocaine-seeking behaviors and self administration of cocaine [79].

With regards to feeding behavior, SB-334867 did not affect self administration of food but almorexant significantly decreased food-maintained responding [78]. On the other hand, a different study reported that SB-334867 reduced food intake in rodents after an overnight fast [80]. Further studies evaluating the effect of hypocretin antagonists on feeding behaviors and obesity are needed.

Almorexant has been shown to attenuate the carbon dioxide response by 26% in wakefulness during the dark period of the diurnal cycle in rats. This decrease was similar to the level observed during NREM sleep in the light period; therefore, the authors of this publication suggest that the sleep–wake difference in carbon dioxide response may be related to hypocretin. Almorexant also decreased the number of sighs and

post-sigh apneas in this study during the light and dark periods [81]. These data compliment prior data discussed showing that hypocretin knockout mice have attenuated central chemoreception [48].

Suvorexant

Suvorexant (MK-4305, [(7R)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl]methanone), is currently under development by Merck. In late 2008, Merck reported a dose-dependent decrease in latency to persistent sleep (LPS) and WASO in a Phase I trial with suvorexant in healthy human subjects [107]. There was no significant impact on SWS or REM activity [107].

In 2010, Merck completed two Phase I, open-labeled, nonrandomized, single-dose studies investigating the pharmacokinetics of suvorexant in patients with either renal insufficiency or hepatic insufficiency [108,109]. No published data are available from these open-labeled studies.

In 2011, Merck launched two Phase I trials investigating the effects of suvorexant on respiratory function in subjects with chronic obstructive pulmonary disease and obstructive sleep apnea [110]. As discussed above, preclinical data suggest that the hypocretin system influences ventilation. Furthermore, rats administered almorexant demonstrate attenuated central chemoreception while awake in the active period of the diurnal cycle [81]. These findings are probably partly responsible for Merck launching a study to evaluate the effects of suvorexant compared with placebo on the respiratory function in subjects with chronic obstructive pulmonary disease, with a primary outcome of mean oxygen saturation (SaO_2) throughout the night and percentage time of total sleep during which SaO_2 is <90, 85 and 80%. Secondary measures include mean apnea/hypopnea index and mean SaO_2 for different sleep stages [110]. The other completed, Phase I, randomized, double-blinded, placebo-controlled trial evaluated the effects of suvorexant on respiratory function in subjects with mild to moderate obstructive sleep apnea. No public data have been disclosed from these two clinical trials.

A Phase I, randomized, double-blinded, crossover assignment trial evaluating the next-day residual effects of multiple doses of suvorexant (20 and 40 mg) on highway driving performance in healthy, nonelderly participants (21–64 years of age) was recently completed but no public data are available [111]. The primary outcome measure is the standard deviation of lateral position on highway driving between MK-4305 and placebo but there is also a secondary measure comparing zopiclone (7.5 mg) with placebo.

A Phase IIb, multicenter, randomized, double-blinded, placebo-controlled crossover dose-ranging study was completed in late 2009 evaluating the safety and efficacy of suvorexant (10, 20, 40 and 80 mg) in subjects (n = 492) with primary insomnia [112]. The primary end point was improvement in sleep efficiency compared with placebo as measured by two PSGs at night one and at the end of 4 weeks of treatment. The secondary end points, also measured by PSG, were improvement in WASO and LPS (or the delay in time to persistent sleep) compared with placebo on night one and at the end of 4 weeks of treatment. The drug was well tolerated and compared with placebo at night one and at the end of 4 weeks significantly improved sleep efficiency (range: 4.7–12.2% depending upon dose and PSG) and significantly reduced WASO (range: -22.9 to -37.9 min depending upon dose and PSG) at all doses. Although this study was not powered to evaluate sleep onset, a significant improvement in LPS was observed at both night one (-21.3 min) and at the end of 4 weeks (-10.7 min) but only with the 80-mg dose [112]. Recall that the higher doses of almorexant in healthy human subjects were also required to significantly improve sleep latency [66]. The most common adverse experiences occurring in patients treated with suvorexant (incidence ≥3% for one or more doses studied) were upper respiratory tract infection, urinary tract infection, increase in alanine aminotransferase and creatinine phosphokinase, dizziness, drowsiness upon awakening, headache, sedation, somnolence and vivid dreams [112].

Merck recently completed a Phase III trial evaluating the safety and efficacy of suvorexant compared with placebo in elderly (≥65 years of age) and nonelderly subjects (≤65 years of age) over 3 months with an optional 3-month extension period [113,114]. There are no public data from this trial.

There is also a completed Phase III multicenter, randomized, double-blinded, placebo-controlled trial that evaluated the long-term safety (for up to 14 months) of suvorexant with primary outcomes looking for patients who experience narcolepsy-like events, including cataplexy; complex sleep-related behavior; propensity for falls; and suicidal ideation and/or behaviors [103]. No published data are available for this study. There are no published, human clinical trials that specifically monitor for cataplexy so these results are greatly anticipated.

SB-649868

SB-649868 is a dual receptor antagonist under development by GlaxoSmithKline that had undisclosed preclinical safety concerns in late 2007 in laboratory rats, resulting in the withdrawal of a Phase I trial [115]. They

have since completed several Phase I trials investigating a variety of parameters including cardiac function, neurologic effects (e.g., evaluating Romberg/heel-to-toe testing), effects of repeat doses of SB-649868 on pharmacokinetics of simvastatin and atorvastatin and interactions with CYP3A4 [116,117]. A Phase II, multicenter, randomized, double-blinded, placebo-controlled, crossover study to evaluate the effects of SB-649868 (10, 30 and 60 mg) on sleep continuity, PSG sleep recordings, subjective sleep assessment and daytime cognitive function in subjects (age 18 – 64 years of age) with primary insomnia for at least 3 months, has been completed but there are no published data.

In a Phase I, single-blinded, randomized, placebo-controlled, single-dose study of SB-649868 (30 mg and 60 mg) administered 2 h before bedtime in healthy volunteers (PSG cohort n = 20), LPS was 15 and 16 min lower ($p < 0.001$) at 30- and 60-mg doses compared with placebo, respectively; TST at 30- and 60-mg doses improved by 27 and 31 min compared with placebo, respectively; WASO did not differ significantly from placebo; and, most importantly, one subject on placebo, one subject on SB-649868 30 mg and seven subjects on SB-649868 60 mg experienced a sleep-onset REM, which the authors defined as a REM latency less than 20 min. In addition, SB-649868 did not significantly affect sleep stages 1, 2 and SWS except for a significant reduction in SWS after the 60-mg dose. No differences in performance on the digital symbol substitution teast (DSST) or verbal learning memory test 10.5 h after SB-649868 (30 or 60 mg) compared with placebo. However, in a different cohort of this study, 2 h after the administration of SB-649868 (30 and 60 mg), which coincides with peak drug level, there was a decrease in cognitive impairment on DSST [82].

Another Phase I, single-site, double-blinded, double-dummy, randomized, placebo-controlled, four-way crossover study was recently published comparing the effects of a single oral dose of zolpidem (10 mg dosed at 22:30, lights off at 23:00) and SB-649868 (10 or 30 mg dosed at 21:30, lights off at 23:00) on healthy male volunteers (n = 44; 18–55 years of age) in a noise-induced situational-insomnia model [83]. The primary outcome was TST measured across four treatment sessions using routine PSG with each session comprising an adaptation night and a treatment night. Data were analyzed according to Rechtschaffen and Kales criteria [70]. A 7-day wash-out period was included between each session during which subjects were encouraged to maintain a 23:00–07:00 sleep pattern. Compliance with this schedule was monitored with actigraphy. Secondary measures included effects of SB-649868 on daytime cognitive function performed 30 min after waking up for each session night. Other sleep parameters included spectral analysis, subjective sleep quality and safety and pharmacokinetics of the two medications [83].

Compared with placebo, TST was significantly increased by 16.8 and 30.7 min for SB-649868 10 and 30 mg, respectively, while zolpidem only increased TST by 11 min. Compared with zolpidem, SB-6496868 30 mg significantly increased TST by 20 min. Although not a primary outcome, the LPS was significantly reduced by 8.5 and 17.4 min after 10 and 30 mg of SB-649868, respectively. Zolpidem did not have a significant effect on LPS. WASO was only significantly reduced in the SB-649868 group by 14.7 min. Only the SB-649868 30-mg group reported significant improvement in subjective sleep latency and TST compared with placebo [83].

With regards to sleep architecture, sleep-onset REM latencies – defined by the authors as the difference between sleep onset and first epoch of REM sleep of ≤15 min – were seen in one subject after the 10-mg dose and two subjects after the 30-mg dose of SB-649868, but none in the placebo or zolpidem group, and overall REM-sleep latency was significantly reduced, compared with placebo, with both doses of SB-649868 (-20.1 and -34 min for 10 and 30 mg, respectively). SB-649868 at 30 mg significantly increased percentage of time spent in REM sleep (+2.3%) while zolpidem significantly decreased the time (-1.4%). While SB-649868 significantly affected REM-sleep parameters, it did not significantly affect percentage of time in SWS over the entire night, whilst zolpidem did significantly affect it by 5.2%. In summary, based upon secondary outcome measures, SB-649868 – but not zolpidem – increased REM sleep parameters while zolpidem – but not SB-649868 – increased duration of SWS in these healthy volunteers in a noise-induced situational-insomnia model [83].

After administration of SB-649868 30 mg, one subject reported mild hallucinations starting 90 min after administration that persisted for 2 h, but was able to complete the trial. The authors do not report if this subject had a sleep-onset REM period. One other subject had severe somnolence after SB-649868 30 mg and this adverse event was deemed by the authors to be of severe intensity and related to SB-649868. In the SB-649868 group, the mean number of lapses (time >500 ms) on psychomotor vigilance testing, which was performed 30 min after waking up, was significantly greater than placebo. All other active treatment doses did not differ significantly from placebo on both the psychomotor vigilance testing and DSST [83]. One should be reminded in the interpretation of these data that this study was performed in healthy males using a traffic noise model of situational insomnia, not

in patients with chronic insomnia.

Overall, adverse effects were similar to placebo except somnolence and disturbed attention was reported by more than twice as many subjects after SB-649868 30 mg, while headache was reported by more than twice as many subjects taking placebo compared with active treatments [83]. This is intriguing in light of the recently disclosed trial by Merck investigating the efficacy of MK-6096 in migraine prophylaxis [118]. No clinically relevant changes were seen on vital signs or 12-lead ECG [83]. Based on preclinical data and past genetic studies showing a relationship between hypocretin and blood pressure, future hypocretin antagonist trials might consider continuous blood pressure monitoring rather than isolated cuff-based measures to evaluate for any changes in dipper versus non-dipper status.

MK-6096

MK-6096, under development by Merck, has completed a Phase IIb, multicenter, randomized, double-blinded, placebo-controlled, crossover study using PSG to evaluate the safety and efficacy in patients with primary insomnia [119]. There are no publically available data on this study but published preclinical data demonstrate very promising sleep-promoting effects in rats and canines [64]. Interestingly, Merck recently launched three Phase IIa studies investigating the safety and efficacy of MK-6096 in the following conditions: painful diabetic neuropathy, adjunctive treatment for major depressive disorder, and migraine prophylaxis in patients with episodic migraines [118,120,121]. These are the first clinical trials to investigate the effects of a hypocretin antagonist on disorders other than insomnia.

Future perspective

Clinical investigation of hypocretin antagonists in the treatment of other sleep disorders such as circadian rhythm disorders (e.g., jet lag, shift-work disorder and delayed sleep-phase syndrome) are very promising, assuming the pharmaceutical industry can demonstrate favorable short- and long-term safety profiles. Hypocretin antagonists affect sleep architecture (e.g., increased REM-sleep duration and latency) differently compared with traditional benzodiazepines, which reduce both SWS and REM sleep. The effects upon sleep appear dependent upon both the type of

Executive summary

Loss of hypocretin-producing neurons in narcolepsy with cataplexy

- Narcolepsy is a syndrome associated with excessive daytime sleepiness, cataplexy, sleep paralysis, hallucinations and disrupted sleep. Other associated features include altered metabolic, neuroendocrine, autonomic and thermoregulatory control.

Characterization of hypocretin neuropeptides & receptors in humans

- There are two hypocretin neuropeptides (HCRT-1 and -2) and two hypocretin receptors (HCRT-1 receptor and HCRT-2 receptor).
- HCRT-1 receptor has a high affinity for HCRT-1 while HCRT-2 receptor has equal affinity for HCRT-1 and -2.
- Hypocretin receptors are differentially expressed in the CNS and found in the periphery.

Physiologic implications of the hypocretin system

- The hypocretin system helps regulate the sleep–wake cycle and the receptors may influence wakefulness, non-rapid eye movement and rapid eye movement sleep differently.
- Hypocretin system may influence other physiologic functions including feeding behavior, the autonomic nervous system, reward and addiction pathways, ventilation, pain pathways and the pituitary–hypothalamic–adrenal axis.
- Downstream effects of hypocretin neuropeptides require further investigation.

Hypocretin antagonists in the treatment of insomnia: preclinical & clinical data

- Selective and dual hypocretin antagonists have been developed and preclinical data suggest that selective hypocretin antagonists may promote sleep more effectively.
- Clinical trials to date are limited to the following dual hypocretin receptor antagonists: almorexant, suvorexant, SB-649868 and MK-6096.
  - Almorexant development was discontinued in early 2011 based upon undisclosed data in spite of a Phase III trial showing superiority over placebo on both objective and subjective wake after sleep onset in chronic primary insomnia subjects.
  - Suvorexant has completed a Phase III trial but there are no published data. A Phase IIb trial showed significant improvements in sleep efficiency and decreased wake after sleep onset. No active trials are currently underway.
  - SB-649868 has published data from Phase I trial demonstrating significant improvements in total sleep time in a situational model for insomnia. A Phase II trial has been completed but there are no published data.
  - MK-6096 has completed a Phase I trial but no published data are available. They recently launched several other trials investigating safety and efficacy in the treatment of migraine prophylaxis, painful diabetic neuropathy and depression.

hypocretin antagonist (selective vs dual) and the time of day administered. This new compound class provides a novel tool to investigate and better understand the relative importance of different sleep stages as well as REM-related phenomenon, such as cataplexy, sleep hallucinations and sleep paralysis. Clinical features of narcolepsy with hypocretin deficiency and preclinical data suggest that the hypocretin system influences many other physiological processes, such as reward and addiction, metabolism, thermoregulation, motor regulation and autonomic control. The trials recently launched investigating the efficacy of a hypocretin antagonist in the treatment of migraines, depression and pain, strongly suggest that many other indications for hypocretin antagonists will be pursued in the coming years. Therapeutic trials investigating the role of selective hypocretin antagonists in disorders such as drug addiction, ventilation, or metabolism might be in the pipeline soon. Future research into the downstream effects of the hypocretin system may lead to more specific therapies for sleep disorders and beyond.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:

- ■ of interest
- ■■ of considerable interest

1 Crocker A, Espana RA, Papadopoulou M *et al.* Concomitant loss of dynorphin, NARP, and orexin in narcolepsy. *Neurology* 65(8), 1184–1188 (2005).

2 Peyron C, Faraco J, Rogers W *et al.* A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat. Med.* 6(9), 991–997 (2000).

3 Thannickal TC, Moore RY, Nienhuis R *et al.* Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 27(3), 469–474 (2000).

4 Mignot E, Lammers GJ, Ripley B *et al.* The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch. Neurol.* 59(10), 1553–1562 (2002).

5 Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* 355(9197), 39–40 (2000).

■ **Details the finding of hypocretin deficiency in human narcolepsy.**

6 Nishino S, Mignot E. Narcolepsy and cataplexy. *Handb. Clin. Neurol.* 99, 783–814 (2011).

7 Mayer G, Hellmann F, Leonhard E, Meier-Ewert K. Circadian temperature and activity rhythms in unmedicated narcoleptic patients. *Pharmacol. Biochem. Behav.* 58(2), 395–402 (1997).

8 Sachs C, Kaijser L. Autonomic control of cardiovascular reflexes in narcolepsy. *J. Neurol. Neurosurg. Psychiatry* 43(6), 535–539 (1980).

9 Dauvilliers Y, Pennestri MH, Whittom S, Lanfranchi PA, Montplaisir JY. Autonomic response to periodic leg movements during sleep in narcolepsy-cataplexy. *Sleep* 34(2), 219–223 (2011).

10 Dauvilliers Y, Pennestri MH, Petit D, Dang-Vu T, Lavigne G, Montplaisir J. Periodic leg movements during sleep and wakefulness in narcolepsy. *J. Sleep Res.* 16(3), 333–339 (2007).

11 Guilleminault C, Eldridge F, Dement WC. Insomnia, narcolepsy, and sleep apneas. *Bull. Physiopathol. Respir.* 8(5), 1127–1138 (1972).

12 Peyron C, Tighe DK, van den Pol AN *et al.* Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.* 18(23), 9996–10015 (1998).

13 de Lecea L, Kilduff TS, Peyron C *et al.* The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl Acad. Sci. USA* 95(1), 322–327 (1998).

■■ **Details the codiscovery of hypocretin neuropeptides.**

14 Sakurai T, Amemiya A, Ishii M *et al.* Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92(4), 573–585 (1998).

■■ **Details the codiscovery of hypocretin neuropeptides.**

15 Yoshida Y, Fujiki N, Maki RA, Schwarz D, Nishino S. Differential kinetics of hypocretins in the cerebrospinal fluid after intracerebroventricular administration in rats. *Neurosci. Lett.* 346(3), 182–186 (2003).

16 Kastin AJ, Akerstrom V. Orexin A but not orexin B rapidly enters brain from blood by simple diffusion. *J. Pharmacol. Exp. Ther.* 289(1), 219–223 (1999).

17 Prokai L. Peptide drug delivery into the central nervous system. *Prog. Drug Res.* 51, 95–131 (1998).

18 Wu MF, Nienhuis R, Maidment N, Lam HA, Siegel JM. Role of the hypocretin (orexin) receptor 2 (HCRT-r2) in the regulation of hypocretin level and cataplexy. *J. Neurosci.* 31(17), 6305–6310 (2011).

19 Mignot E, Nishino S, Sharp LH *et al.* Heterozygosity at the canarc-1 locus can confer susceptibility for narcolepsy: induction of cataplexy in heterozygous asymptomatic dogs after administration of a combination of drugs acting on monoaminergic and cholinergic systems. *J. Neurosci.* 13(3), 1057–1064 (1993).

20 Sakurai T, Mieda M. Connectomics of orexin-producing neurons: interface of systems of emotion, energy homeostasis and arousal. *Trends Pharmacol. Sci.* 32(8), 451–462 (2011).

21 Borbely AA. A two process model of sleep regulation. *Hum. Neurobiol.* 1(3), 195–204 (1982).

22 Taheri S, Sunter D, Dakin C *et al.* Diurnal variation in orexin A immunoreactivity and preproorexin mRNA in the rat central nervous system. *Neurosci. Lett.* 279(2), 109–112 (2000).

23 Yoshida Y, Fujiki N, Nakajima T *et al.* Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light–dark cycle and sleep–wake activities. *Eur. J. Neurosci.* 14(7), 1075–1081 (2001).

24 Zeitzer JM, Buckmaster CL, Parker KJ, Hauck CM, Lyons DM, Mignot E. Circadian and homeostatic regulation of hypocretin in a primate model: implications for the consolidation of wakefulness. *J. Neurosci.* 23(8), 3555–3560 (2003).

25 Martinez GS, Smale L, Nunez AA. Diurnal and nocturnal rodents show rhythms in orexinergic neurons. *Brain Res.* 955(1–2), 1–7 (2002).

26 Fujiki N, Yoshida Y, Ripley B, Honda K, Mignot E, Nishino S. Changes in CSF hypocretin-1 (orexin A) levels in rats across

24 hours and in response to food deprivation. *Neuroreport* 112(5), 993–997 (2001).

27 Mieda M, Hasegawa E, Kisanuki YY, Sinton CM, Yanagisawa M, Sakurai T. Differential roles of orexin receptor-1 and -2 in the regulation of non-REM and REM sleep. *J. Neurosci.* 31(17), 6518–6526 (2011).

28 Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M. Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. *Proc. Natl Acad. Sci. USA* 101(13), 4649–4654 (2004).

29 Huang ZL, Qu WM, Li WD *et al.* Arousal effect of orexin A depends on activation of the histaminergic system. *Proc. Natl Acad. Sci. USA* 98(17), 9965–9970 (2001).

30 Yamanaka A, Tsujino N, Funahashi H *et al.* Orexins activate histaminergic neurons via the orexin 2 receptor. *Biochem. Biophys. Res. Commun.* 290(4), 1237–1245 (2002).

31 Hondo M, Nagai K, Ohno K *et al.* Histamine-1 receptor is not required as a downstream effector of orexin-2 receptor in maintenance of basal sleep/wake states. *Acta Physiol.* 198(3), 287–294 (2010).

32 Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* 450(7168), 420–424 (2007).

33 Willie JT, Chemelli RM, Sinton CM *et al.* Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. *Neuron* 38(5), 715–730 (2003).

34 Espana RA, Scammell TE. Sleep neurobiology from a clinical perspective. *Sleep* 34(7), 845–858 (2011).

35 Schuld A, Hebebrand J, Geller F, Pollmacher T. Increased body-mass index in patients with narcolepsy. *Lancet* 355(9211), 1274–1275 (2000).

36 Honda Y, Doi Y, Ninomiya R, Ninomiya C. Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients. *Sleep* 9(1), 254–259 (1986).

37 Dahmen N, Bierbrauer J, Kasten M. Increased prevalence of obesity in narcoleptic patients and relatives. *Eur. Arch. Psychiatry Clin. Neurosci.* 251(2), 85–89 (2001).

38 Hara J, Yanagisawa M, Sakurai T. Difference in obesity phenotype between orexin-knockout mice and orexin neuron-deficient mice with same genetic background and environmental conditions. *Neurosci. Lett.* 380(3), 239–242 (2005).

39 Yamanaka A, Beuckmann CT, Willie JT *et al.* Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38(5), 701–713 (2003).

40 Bonnavion P, de Lecea L. Hypocretins in the control of sleep and wakefulness. *Curr. Neurol. Neurosci. Rep.* 10(3), 174–179 (2010).

41 Smith BN, Davis SF, Van Den Pol AN, Xu W. Selective enhancement of excitatory synaptic activity in the rat nucleus tractus solitarius by hypocretin 2. *Neuroscience* 115(3), 707–714 (2002).

42 Young JK, Wu M, Manaye KF *et al.* Orexin stimulates breathing via medullary and spinal pathways. *J. Appl. Physiol.* 98(4), 1387–1395 (2005).

43 Shirasaka T, Nakazato M, Matsukura S, Takasaki M, Kannan H. Sympathetic and cardiovascular actions of orexins in conscious rats. *Am. J. Physiol.* 275(6), R1780–R1785 (1999).

44 Zhang W, Fukuda Y, Kuwaki T. Respiratory and cardiovascular actions of orexin-A in mice. *Neurosci. Lett.* 385(2), 131–136 (2005).

45 Schwimmer H, Stauss HM, Abboud F, Nishino S, Mignot E, Zeitzer JM. Effects of sleep on the cardiovascular and thermoregulatory systems: a possible role for hypocretins. *J. Appl. Physiol.* 109(4), 1053–1063 (2010).

46 Williams RH, Jensen LT, Verkhratsky A, Fugger L, Burdakov D. Control of hypothalamic orexin neurons by acid and CO_2. *Proc. Natl Acad. Sci. USA* 104(25), 10685–10690 (2007).

47 Sunanaga J, Deng BS, Zhang W, Kanmura Y, Kuwaki T. CO_2 activates orexin-containing neurons in mice. *Respir. Physiol. Neurobiol.* 166(3), 184–186 (2009).

48 Nakamura A, Zhang W, Yanagisawa M, Fukuda Y, Kuwaki T. Vigilance state-dependent attenuation of hypercapnic chemoreflex and exaggerated sleep apnea in orexin knockout mice. *J. Appl. Physiol.* 102(1), 241–248 (2007).

49 Espana RA, Oleson EB, Locke JL, Brookshire BR, Roberts DC, Jones SR. The hypocretin-orexin system regulates cocaine self administration via actions on the mesolimbic dopamine system. *Eur. J. Neurosci.* 31(2), 336–348 (2010).

50 Narita M, Nagumo Y, Hashimoto S *et al.* Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J. Neurosci.* 26(2), 398–405 (2006).

51 Boutrel B, Kenny PJ, Specio SE *et al.* Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. *Proc. Natl Acad. Sci. USA* 102(52), 19168–19173 (2005).

52 Bingham S, Davey PT, Babbs AJ *et al.* Orexin-A, an hypothalamic peptide with analgesic properties. *Pain* 92(1–2), 81–90 (2001).

53 Arihara Z, Takahashi K, Murakami O *et al.* Immunoreactive orexin-A in human plasma. *Peptides* 22(1), 139–142 (2001).

54 Karteris E, Chen J, Randeva HS. Expression of human preproorexin and signaling characteristics of orexin receptors in the male reproductive system. *J. Clin. Endocrinol. Metab.* 89(4), 1957–1962 (2004).

55 Blanco M, Gallego R, Garcia-Caballero T, Dieguez C, Beiras A. Cellular localization of orexins in human anterior pituitary. *Histochem. Cell. Biol.* 120(4), 259–264 (2003).

56 Digby JE, Chen J, Tang JY, Lehnert H, Matthews RN, Randeva HS. Orexin receptor expression in human adipose tissue: effects of orexin-A and orexin-B. *J. Endocrinol.* 191(1), 129–136 (2006).

57 Nakabayashi M, Suzuki T, Takahashi K *et al.* Orexin-A expression in human peripheral tissues. *Mol. Cell. Endocrinol.* 205(1–2), 43–50 (2003).

58 Hagan JJ, Leslie RA, Patel S *et al.* Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc. Natl Acad. Sci. USA* 196(19), 10911–10916 (1999).

59 Kuru M, Ueta Y, Serino R *et al.* Centrally administered orexin/hypocretin activates HPA axis in rats. *Neuroreport* 11(9), 1975–1980 (2000).

60 Furuta M, Mitsushima D, Shinohara K, Kimura F, Funabashi T. Food availability affects orexin A/hypocretin-1-induced inhibition of pulsatile luteinizing hormone secretion in female rats. *Neuroendocrinology* 91(1), 41–47 (2010).

61 American Academy of Sleep Medicine. *ICSD-2-International Classification of Sleep Disorders (2nd Edition).* American Academy of Sleep Medicine, Westchester, IL, USA (2005).

62 Ohayon MM. Epidemiology of insomnia: what we know and what we still need to learn. *Sleep. Med. Rev.* 6(2), 97–111 (2002).

63 Coleman PJ, Renger JJ. Orexin receptor antagonists: a review of promising compounds

patented since 2006. *Expert Opin. Ther. Pat.* 20(3), 307–324 (2010).

64 Winrow CJ, Gotter AL, Cox CD *et al.* Pharmacological characterization of MK-6096 – a dual orexin receptor antagonist for insomnia. *Neuropharmacology* 62(2), 978–987 (2012).

65 Morin CM, Vallieres A, Guay B *et al.* Cognitive behavioral therapy, singly and combined with medication, for persistent insomnia: a randomized controlled trial. *JAMA* 301(19), 2005–2015 (2009).

66 Brisbare-Roch C, Dingemanse J, Koberstein R *et al.* Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat. Med.* 13(2), 150–155 (2007).

■■ **One of the few published clinical trials on dual hypocretin antagonists in humans.**

67 Steiner MA, Lecourt H, Strasser DS, Brisbare-Roch C, Jenck F. Differential effects of the dual orexin receptor antagonist almorexant and the GABA(A)-α1 receptor modulator zolpidem, alone or combined with ethanol, on motor performance in the rat. *Neuropsychopharmacology* 36(4), 848–856 (2010).

68 Hoever P, de Haas S, Winkler J *et al.* Orexin receptor antagonism, a new sleep-promoting paradigm: an ascending single-dose study with almorexant. *Clin. Pharmacol. Ther.* 87(5), 593–600 (2010).

■■ **One of the few published clinical trials on dual hypocretin antagonists in humans.**

69 Littner MR, Kushida C, Wise M *et al.* Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. *Sleep* 28(1), 113–121 (2005).

70 Rechtschaffen A, Kales A. *A Manual of standardized terminology, techniques, and scoring system for sleep stages of human subjects.* Brain Information Service/Brain Research Institute, University of California, CA, USA (1968).

71 Aldrich MS, Rogers AE. Exacerbation of human cataplexy by prazosin. *Sleep* 12(3), 254–256 (1989).

72 Chemelli RM, Willie JT, Sinton CM *et al.* Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 98(4), 437–451 (1999).

73 Schutte-Rodin S, Broch L, Buysse D, Dorsey C, Sateia M. Clinical guideline for the evaluation and management of chronic insomnia in adults. *J. Clin. Sleep. Med.* 4(5), 487–504 (2008).

74 Dietrich H, Jenck F. Intact learning and memory in rats following treatment with the dual orexin receptor antagonist almorexant. *Psychopharmacology* 212(2), 145–154 (2010).

75 Willie JT, Takahira H, Shibahara M *et al.* Ectopic overexpression of orexin alters sleep/wakefulness states and muscle tone regulation during REM sleep in mice. *J. Mol. Neurosci.* 43(2), 155–161 (2011).

76 Dugovic C, Shelton JE, Aluisio LE *et al.* Blockade of orexin-1 receptors attenuates orexin-2 receptor antagonism-induced sleep promotion in the rat. *J. Pharmacol. Exp. Ther.* 330(1), 142–151 (2009).

■■ **Proposes that selective rather than dual hypocretin antagonists are more effective at promoting sleep.**

77 Smith MI, Piper DC, Duxon MS, Upton N. Evidence implicating a role for orexin-1 receptor modulation of paradoxical sleep in the rat. *Neurosci. Lett.* 341(3), 256–258 (2003).

78 LeSage MG, Perry JL, Kotz CM, Shelley D, Corrigall WA. Nicotine self administration in the rat: effects of hypocretin antagonists and changes in hypocretin mRNA. *Psychopharmacology* 209(2), 203–212 (2010).

79 Smith RJ, See RE, Aston-Jones G. Orexin/hypocretin signaling at the orexin 1 receptor regulates cue-elicited cocaine-seeking. *Eur. J. Neurosci.* 30(3), 493–503 (2009).

80 Haynes AC, Jackson B, Chapman H *et al.* A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul. Pept.* 96(1–2), 45–51 (2000).

81 Li A, Nattie E. Antagonism of rat orexin receptors by almorexant attenuates central chemoreception in wakefulness in the active period of the diurnal cycle. *J. Physiol.* 588(15), 2935–2944 (2010).

82 Bettica P, Nucci G, Pyke C *et al.* Phase I studies on the safety, tolerability, pharmacokinetics and pharmacodynamics of SB-649868, a novel dual orexin receptor antagonist. *J. Psychopharmacol.* doi:10.1177/0269881111408954 (2011) (Epub ahead of print).

83 Bettica P, Squassante L, Groeger JA, Gennery B, Winsky-Sommerer R, Dijk DJ. Differential Effects of a dual orexin receptor antagonist (SB-649868) and zolpidem on sleep initiation and consolidation, SWS, REM Sleep and EEG power spectra in a model of situational insomnia. *Neuropsychopharmacology* 37(5), 1224–1233 (2012).

■■ **One of the few published trials on dual hypocretin antagonists in humans and the first disclosed clinical trial investigating efficacy of a hypocretin antagonist in disorders other than insomnia.**

84 Cox CD, Breslin MJ, Whitman DB *et al.* Discovery of the dual orexin receptor antagonist ([7R]-4-[5-chloro-1,3-benzoxazol-2-yl]-7-methyl-1,4-diazepan-1-yl)(5-methyl-2-[2H -1,2,3-triazol-2-yl] phenyl) methanone (MK-4305) for the treatment of insomnia. *J. Med. Chem.* 53(14), 5320–5332 (2010).

85 Coleman PJ, Schreier JD, Cox CD *et al.* Discovery of ([2R,5R]-5-[([5-Fluoropyridin-2-yl]oxy)methyl]-2-methylpiperidin-1-yl) (5-methyl-2 -[pyrimidin-2-yl]phenyl) methanone (MK-6096): a dual orexin receptor antagonist with potent sleep-promoting properties. *ChemMedChem* 7(3), 415–424 (2012).

86 Bergman JM, Roecker AJ, Mercer SP *et al.* Proline bis-amides as potent dual orexin receptor antagonists. *Bioorg. Med. Chem. Lett.* 18(4), 1425–1430 (2008).

87 McAtee LC, Sutton SW, Rudolph DA *et al.* Novel substituted 4-phenyl-dioxanes: potent and selective orexin receptor 2 (OX[2]R) antagonists. *Bioorg. Med. Chem. Lett.* 14(16), 4225–4229 (2004).

88 Whitman DB, Cox CD, Breslin MJ *et al.* Discovery of a potent, CNS-penetrant orexin receptor antagonist based on an n,n-disubstituted-1,4-diazepane scaffold that promotes sleep in rats. *ChemMedChem* 4(7), 1069–1074 (2009).

89 Gerrard P, Porter R, Holland V *et al.* Preclinical pharmacology of SB-649868: a novel orexin Ox1/Ox2 receptor antagonist possessing potent hypnotic activity in rodents and primates. *Sleep* 32:A42 (2009).

90 Langmead CJ, Jerman JC, Brough SJ, Scott C, Porter RA, Herdon HJ. Characterisation of the binding of (3H)-SB-674042, a novel nonpeptide antagonist, to the human orexin-1 receptor. *Br. J. Pharmacol.* 141(2), 340–346 (2004).

91 Malherbe P, Borroni E, Gobbi L *et al.* Biochemical and behavioural characterization of EMPA, a novel high-affinity, selective antagonist for the OX(2) receptor. *Br. J. Pharmacol.* 156(8), 1326–1341 (2009).

■ Websites

101 Actelion and GlaxoSmithKline discontinue clinical development of almorexant (2011). www1.actelion.com/en/journalists/news-archive.page?newsId=1483135

■■ **First hypocretin antagonist to show**

superiority over placebo in subjective and objective wake after sleep onset, but development discontinued due to the safety and clinical profile of the compound.

102 Merck. US Securities and Exchange Commission Form 10-K Merck & Co., Inc. (2012). www.merck.com/investors/financials/form-10-k-2011.pdf

■■ **Merck plans to file a new drug application with the US FDA for suvorexant.**

103 A long-term safety study of MK4305 in patients with primary insomnia (2011). www.clinicaltrials.gov/ct2/show/NCT01021813?term=MK-4305&rank=4

104 ACT-078573 in elderly subjects with chronic primary insomnia (2010). www.clinicaltrials.gov/ct2/show/NCT00606593?term=almorexant&rank=4

105 Investigational dual orexin receptor antagonist almorexant meets primary end point in two-week Phase III study in patients suffering from primary insomnia (2011). www1.actelion.com/en/our-company/news-and-events/index.page?newsId=1365361

106 Effect of a hypocretin/orexin antagonist on neurocognitive performance (2011). www.clinicaltrials.gov/ct2/show/NCT01243060?term=almorexant&rank=1

107 Merck 2008 annual business briefing – final transcript (2008). http://library.corporate-ir.net/library/73/731/73184/items/318277/%7B6EFE21B3-D8AB-4426-9C97-1F3D77744843%7D_120908.pdf

108 Pharmacokinetics of MK4305 in patients with hepatic insufficiency (2010). www.clinicaltrials.gov/ct2/show/NCT01043926?term=MK-4305&rank=7

109 Pharmacokinetics of MK4305 in patients with impaired renal function (MK-4305–4023) (2010). www.clinicaltrials.gov/ct2/show/NCT01059851?term=MK-4305&rank=4

110 Effects of MK-4305 (suvorexant) in patients with chronic obstructive pulmonary disease (MK-4305–4032 AM2) (2011). www.clinicaltrials.gov/ct2/show/NCT01293006?term=MK-4305&rank=5

111 A study to evaluate next day effects of MK-4305 on driving performance (MK-4305–4035) (2011). www.clinicaltrials.gov/ct2/show/NCT01311882?term=MK-4305&rank=1

112 Merck - results from Phase IIb clinical study of insomnia therapy MK-4305 (2010). www.biopharmamarket.com/@offers/news/view/1762

113 Safety and efficacy study in primary insomnia patients – study A, 4305–4028 (2011). www.clinicaltrials.gov/ct2/show/NCT01097616?term=MK-4305&rank=1

114 Safety and efficacy study in primary insomnia patients – study B (4305–4029) (2011). www.clinicaltrials.gov/ct2/show/NCT01097629?term=MK-4305&rank=2

115 A clinical study to evaluate the pharmacokinetic profile of SB-649868 in elderly and female population (2010). www.clinicaltrials.gov/ct2/show/NCT00534872?term=SB+%E2%80%93+649868&rank=1

116 A single-centre open label study to investigate the effect of repeat doses of SB-649868 on the pharmacokinetics of simvastatin and atorvastatin in healthy male volunteers (2011). www.clinicaltrials.gov/ct2/show/NCT01299597?term=SB-649868&rank=4

117 Study to investigate safety, tolerability, pharmacokinetics and cardiac function after repeat doses of SB-649868 in healthy volunteers (2011). www.clinicaltrials.gov/ct2/show/NCT01030939?term=SB+%E2%80%93+649868&rank=4. Accessed 12/19, 2011

118 A study of the safety and efficacy of MK-6096 for Migraine prophylaxis in participants with episodic migraine (MK-6096–6020) (2012). www.clinicaltrials.gov/ct2/show/NCT01513291?term=MK-6096&rank=1

■■ **First disclosed clinical trial investigating efficacy of a hypocretin antagonist in the treatment of migraine prophylaxis.**

119 Polysomnography Study of MK6096 in patients with primary insomnia (6096–6011) (COMPLETED) (2012). www.clinicaltrials.gov/ct2/show/NCT01021852?term=MK-6096&rank=4

120 Study to Evaluate MK-6096 in the treatment of painful diabetic neuropathy (PDN) in adults (MK-6096–6021) (2012). www.clinicaltrials.gov/ct2/show/NCT01564459?term=MK-6096&rank=3

■■ **First disclosed clinical trial investigating efficacy of a hypocretin antagonist in the treatment of diabetic neuropathy.**

121 Safety and Efficacy of MK-6096 as adjunctive therapy in participants with major depressive disorder and partial response to antidepressant monotherapy (MK-6096–6022 AM1) (2012). www.clinicaltrials.gov/ct2/show/NCT01554176?term=MK-6096&rank=2

■■ **First disclosed clinical trial investigating efficacy of a hypocretin antagonist in the treatment of major depressive disorder.**