Pharmacological Reports 68 (2016) 231–242

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Review article

Emerging role of orexin antagonists in insomnia therapeutics: An update on SORAs and DORAs

Anil Kumar *, Priyanka Chanana, Supriti Choudhary

Pharmacology Division, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Study, Panjab University, Chandigarh, India

ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form 9 September 2015
Accepted 10 September 2015
Available online 28 September 2015

Keywords:
DORAs
Insomnia
Orexin
Orexin receptors
SORAs

ABSTRACT

The pharmacological management of insomnia has lately become a challenge for researchers worldwide. As per the third International Classification of Sleep disorders (ICSD-3) insomnia can be defined as a state with repeated difficulty in sleep initiation, duration, consolidation, or quality that occurs despite adequate opportunity and circumstances for sleep, and results in some form of daytime impairment. The conventional treatments approved for management of insomnia were benzodiazepines (BZDs) (estazolam, quazepam, triazolam, flurazepam and temazepam) and non-BZDs, also known as z-drugs (zaleplon, zolpidem, and eszopiclone), tricyclic antidepressant (TCA) doxepin as well as melatonin agonists, e.g. ramelteon. But the potential of these agents to address sleep problems has been limited due to substantial side effects associated with them like hangover, dependence and tolerance, rebound insomnia, muscular atonia, inhibition of respiratory system, cognitive dysfunctions, and increased anxiety. Recently, orexin neuropeptides have been identified as regulators of transition between wakefulness and sleep and documented to aid an initial transitory effect towards wakefulness by activating cholinergic/monoaminergic neural pathways of the ascending arousal system. This has led to the development of orexin peptides and receptors, as possible therapeutic targets for the treatment of sleep disorders with the advantage of having lesser side effects as compared to conventional treatments. The present review focuses on the orexin peptides and receptors signifying their physiological profile as well as the development of orexin receptor antagonists as novel strategies in sleep medicine.

Contents

Abbreviations: DR, dorsal raphe; DMH, dorsomedial hypothalamus; GPCR, G protein coupled receptor; GABA, gamma amino butyric acid; LDT, laterodorsal tegmental nucleus; LC, locus coeruleus; LPC, lysophosphatydylcholine; NMDA, N-methyl-D-aspartate; NSCC, non-selective cationic channels; OX_1R, Orexin receptor 1; OX_2R, orexin receptor 2; PPT, pedunculopontine nucleus; PUFAs, polyunsaturated fatty acids; VTA, ventral tegmental area; RAS, reticular activating system; VLPO, venterolateral preoptic; 5-HT, 5-hydroxy tryptamine.

* Corresponding author.
E-mail address: kumaruips@yahoo.com (A. Kumar).

http://dx.doi.org/10.1016/j.pharep.2015.09.002
1734-1140/

Introduction

Insufficient sleep is considered as a public health epidemic. Approximately 65 million adults in the USA (36% of total population) complain of poor sleep, and out of these, 25% suffer from insomnia on chronic basis. As per the third International Classification of Sleep disorders (ICSD-3) insomnia can be defined as a state with repeated difficulty in sleep initiation, duration, consolidation, or quality that occurs despite adequate opportunity and circumstances for sleep, and results in some form of daytime impairment [1].

The sleep–wake cycle is controlled by a reciprocal inhibitory interaction between the wakefulness-promoting orexinergic, noradrenergic, serotoninergic, and cholinergic neural systems of the ascending reticular activating system (RAS) and sleep promoting venterolateral preoptic (VLPO) region. The reciprocal inhibitory control between the arousal areas and the VLPO neurons establishes a feedback loop that regulates the sleep wake homeostasis [2,3].

Orexins hold an important role in the wakefulness promoting ascending arousal system by having an excitatory effect on almost every wake promoting neuronal group of RAS. During the past few years many clinical and preclinical studies as well as reviews have focussed on hypothalamic neuropeptides 'orexins' (orexin A and B) for characterising their effects on central nervous system (CNS). Counteracting their initial effects on appetite regulation, recent research has established orexins as the critical modulators of the sleep wake cycle homeostasis [4,5]. These findings have brought about the possibility of development of novel therapeutic agents targeting the orexin cascade (receptors and peptides) for treatment of sleep disorders. These agents in turn were also expected to be associated with lesser adverse effects unlike the conventional treatments for insomnia like benzodiazepine receptor agonists (BzRAs), non benzo-diazepines (e.g. zolpidem, zaleplon), sedating antidepressants such as trazodone, amitriptyline, and doxepin and melatonin and the melatonin agonists. The present review focusses on neuropeptides orexin A and B, their receptor pharmacologies, the role played by them in modulating sleep/wake homeostasis and exploring orexin receptors as therapeutic targets for treating sleep disturbances.

The orexin cascade

Orexin peptides

The history of orexin peptides can be accorded way back to the time when orexins were independently isolated by two different groups of researchers. While one of the groups was exploring hypothalamic neuropeptides, the other was researching upon possible endogenous ligands of the then categorised 'orphan G protein coupled receptors' (GPCR) (ligands to these receptors remain unknown), delineated today as orexin receptor 1 (OX_1R) [7,8]. Both orexin A and B are derived by enzymatic action of convertases, according a post translational modification of a common precursor called prepro-orexin [6,8]. While orexin A is chemically characterised by a 33-amino acid peptide chain, with N-terminal cyclized with a pyroglutamyl residue, two intra-chain disulphide bonds and C-terminal amidation, orexin B is a 28-amino acid, C-terminally amidated linear peptide which probably forms two alpha helices. No doubt, concentration of orexin B in the brain is 2–5 times higher in comparison to orexin A, but the stability of orexin A in the cerebrospinal fluid and blood as well as its behavioural effects are more distinguished as compared to orexin B [4,8].

The orexin peptides are produced by a cluster of orexinergic neurons localised in the perifornical area and the lateral and posterior hypothalamus areas of the brain. The estimated number of these neurons has been found to be around 3000–4000 in rat brains and 70,000 in human brains [5,9]. The orexinergic neurons send extensive input projections to nuclei that regulate arousal and motivation, like the noradrenergic neurons of the locus coeruleus (LC), the histaminergic neurons of the tuberomammillary nucleus (TMN), and the serotonergic neurons of the raphe nuclei as the cholinergic and noncholinergic neurons of the basal forebrain. They also send afferents to the dopaminergic neurons of the ventral tegmental area (VTA) that control wakefulness, attention and REM sleep, the nucleus accumbens, the substantia niagra and VTA regions that control motivation, reward, feeding and locomotion.

These orexin neurons receive a variety of neural signals from different brain areas as well. They receive strong inputs from regions like the amygdala and insular cortex found to be responsible for mediating responses to stress and autonomic tone and the nucleus accumbens and VTA nucleus that regulate reward and motivation supply. Moreover, they are innervated by neurons originating from serotoninergic raphe hypothalamus (DMH), which are responsible for supplying information related to circadian rhythms and the timing of wakefulness. The orexinergic neurons receiving such innervations, exemplify signal information from these diverse inputs, and appropriately promote arousal [5,9].

Orexin receptors

Orexins play an important role in increasing the depolarisation and excitability of the neurons. Their action is mediated *via* two different G protein coupled receptors: the orexin receptor 1 and orexin receptor 2 (OX_1R and OX_2R). The OX_1R is selective for orexin-A while OX_2R is non-selective, showing binding affinities for both orexin-A and B [8,10]. The OX_1R is thought to couple to Gq protein and its excitation causes activation of phospholipase Cβ (PLCβ) pathway, further enabling the release of calcium ions from endoplasmic reticulum and subsequent depolarisation. The intracellular Ca^{2+} concentration also increases by an alternate means, i.e. *via* influx from specific non-selective cationic channels (NSCC) located in the cell membrane that get opened with the help of Gq receptors activation. OX_1R may also activate phospholipase A2 leading to the production of lysophosphatydylcholine (LPC) and polyunsaturated fatty acids (PUFAs), which further mediate Ca^{2+} influx by acting as ligands for the opening of NSCC. This net

increase in intracellular Ca^{2+} concentrations by various routes leads to an environment of neuronal depolarisation and excitation [10].

Unlike OX_1R, the action of OX_2R is accorded by a conjugated Gq and Gi/o protein. In the case of Gi/o protein activation, closing of G protein gated potassium (GIRK-G protein gated inwardly rectifying K^+ channels) channels occurs, which leads to increased neuronal activity by inhibiting the K^+ outflow [10]. Fig. 1 describes the mechanisms involved in orexin receptor signalling in brain cells.

Ancillary to these postsynaptic effects, orexins can also act on presynaptic nerve terminals to aid release of GABA or glutamate, thus generating more complicated myriad of effects [11,12]. As mentioned earlier orexins have the tendency to produce sustained excitability of the neurons. In order to elucidate the plausible mechanism underlying the same, studies have documented that in specific regions like the VTA region, orexins increase the responsiveness of the neurons towards the excitatory effects of glutamate by increasing the number of membranal *N*-methyl-D-aspartate (NMDA) receptors [13,14]. Through the above mechanisms of increased neuronal responsiveness, the orexins are thought to promote sustained excitation of neurons that initiate many aspects of arousal.

The distribution patterns of OX_1R and OX_2R in CNS areas are partially overlapping but largely distinct and complementary. OX_1R is found to be expressed in the prefrontal and infralimbic cortex, hippocampus, amygdala, bed nucleus of the stria terminalis (BST), paraventricular thalamic nucleus, anterior hypothalamus, dorsal raphe (DR), VTA, LC, and laterodorsal tegmental nucleus (LDT)/pedunculopontine nucleus (PPT) [15,16]. The expression sites of OX_2R are at the amygdala, BST, paraventricular thalamic nucleus, DR, VTA, and LDT/PPT [15,16]. OX_2R is also found to be expressed at the arcuate nucleus (Arc), TMN, dorsomedial hypothalamic nucleus (DMH), paraventricular nucleus, lateral hypothalamus, hippocampus, and medial septal nucleus [15,16]. This diversified distribution of orexin receptors across the brain clearly suggests that orexins are likely to play a regulatory control over the CNS and regulate feeding, autonomic control, sleep, memory, as well as the reward system.

Orexinergic signalling: control of sleep and wakefulness homeostasis

Orexin peptides play a number of diverse physiological roles in the body like controlling motivation, feeding, attention, reward, etc. Fig. 2 clearly depicts the array of physiological roles performed by orexins wherein motivating signals are integrated, processed by the afferent nuclei and converted into responses like motivation, reward, locomotion, wakefulness and sympathetic tone through the target nuclei innervations. But the most dominant behavioural effect by orexin action is generated on the sleep wake cycle or specifically arousal.

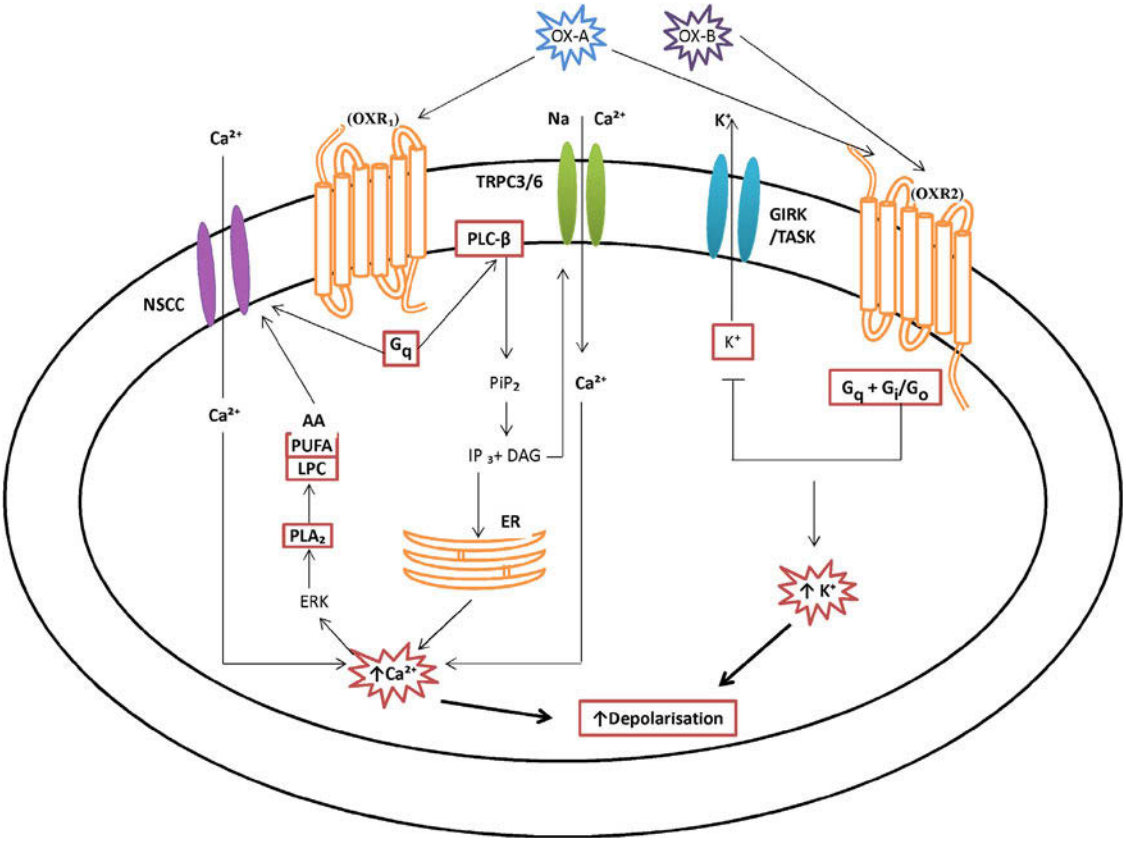

Fig. 1. Orexinergic innervation brings about a sustained excitatory effect on almost all wake promoting encephalic areas. The depolarisation is attributed to various mechanisms which may co-exist in individual neurons. The mechanisms like increased intracellular Ca^{2+} concentration (*via* activation of IP_3 receptors over endoplasmic reticulum aiding Ca^{2+} release as well as influx to replenish intracellular stores *via* ion channels (Non Specific Cation Channels activated *via* PLA_2 intermediation, DAG dependent cation channels especially the TRPC3 and TRPC6 channels) and inhibition of K^+ channels especially the GIRK and TASK channels to impede the efflux of K^+ ions. Through these mechanisms an environment of sustained depolarisation is accorded. AA, arachidonic acid; DAG, diacyl glycerol; ERK, extracellular signal-regulated kinases; GIRK, the G protein-coupled inwardly-rectifying potassium channels; IP_3: inositol triphosphate; LPC, lysoglycerophospholipids; NSCC, Non Specific Cation Channels; OXA and B, orexin A and B; OXR1 and OXR2, orexin receptor 1 and 2; PLA_2, phospholipase A_2; PLC, phospholipase C; PUFA, polyunsaturated fatty acids; TASK, TWIK related acid sensitive K^+ channels; TRPC: transient receptor potential-Canonical K^+ channels, →, activation; ⟞, inhibition.

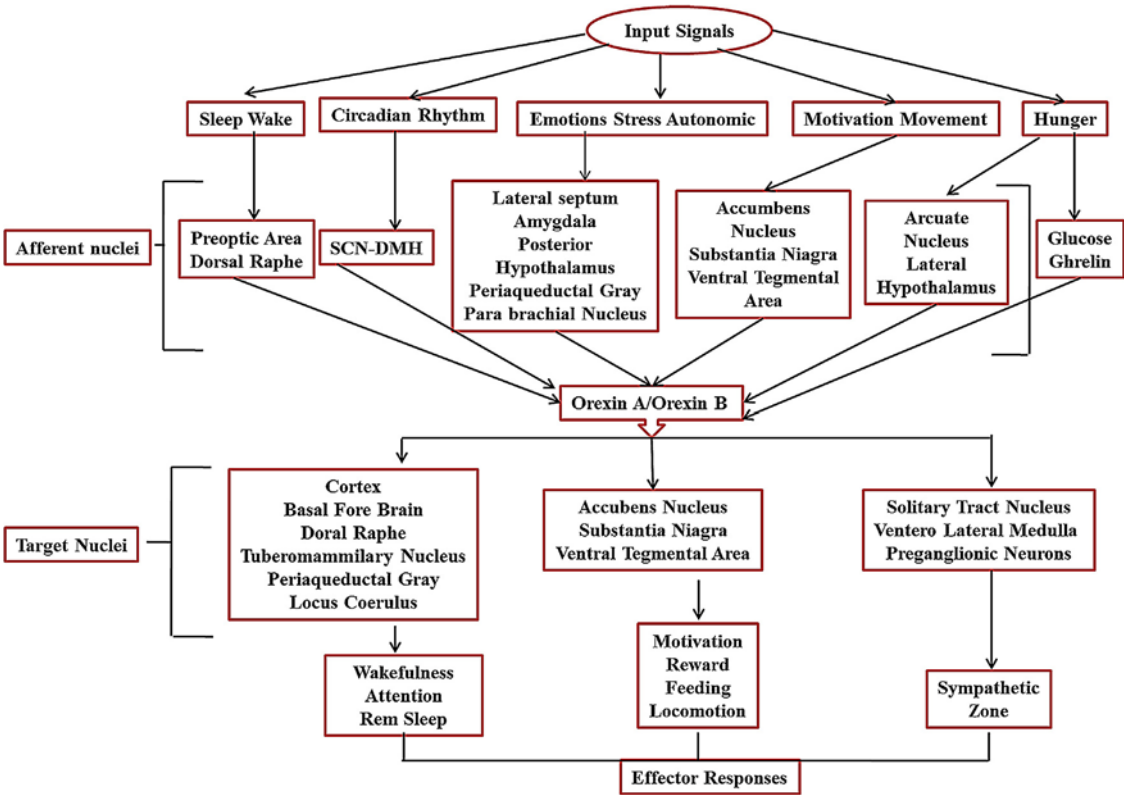

Fig. 2. Describes the plausible sequence of steps involved in the physiological control of orexins over stress, sleep, motivation, hunger, locomotion as well as sympathetic tone. SCN-DMH, suprachiasmatic nucleus, dorsomedial hypothalamus.

Interaction with sleep wake centres

The sleep wake flip flop cycle consists of wake promoting as well as sleep promoting neuronal structures which show contrasting inhibitory functions in order to maintain a homeostasis. While the sleep promoting neurons seem to be located mainly in the VLPO area, the wakefulness promoting areas consists of the LC, TMN, raphe nuclei etc. [17]. Sleep-active VLPO neurons play a pivotol role in initiation of non-rapid-eye-movement (NREM) sleep and maintenance of both NREM and rapid-eye-movement (REM) sleep [18]. VLPO sends inhibitory innervations to wake-promoting neuronal areas producing neurotransmitters, including histamine, noradrenaline, 5-HT, and acetylcholine which precipitate wakefulness [18,19]. The VLPO neurons mostly contain gamma amino butyric acid (GABA) and/or galanin and fire extensively during sleep while during wake they remain quiescent as they are inhibited by wake-active transmitters [20]. These reciprocal interactions of inhibition between the sleep promoting VLPO neurons and the wake promoting monoaminergic neurons (the arousal activating system), constitute the flip–flop switching of the wake/sleep states.

The orexin neurons serve as initiator of the ascending arousal system as they have shown to produce an excitatory effect on almost every wake promoting neuronal group [21]. When VLPO neurons send GABAergic inhibitory projections to orexin neurons, it leads to turning off of orexin projections and thus accords sleep. Also, studies have shown that orexin neurons are strongly inhibited by both a GABAA receptor agonist, muscimol, and GABAB receptor agonist, baclofen [22,23]. It has been documented that orexin neurons discharge immensely during active waking and cease firing during sleep, including the NREM and REM periods, *in vivo* [24]. The noradrenergic cells of the LC, dopaminergic cells of the VTA [25,26], serotonergic cells of the DR [27], and histaminergic cells of the TMN [28], all possess orexin receptors and are activated by orexins *in vitro*. These results suggest that orexin neurons exert an excitatory influence on each of these wake-active neurons to sustain their activity. Also, orexins directly innervate the cholinergic neurons in the BF and the LDT/PPT [29], which also play an important role in regulating arousal [30]. Consequently it can be concluded that orexin neurons are physiologically active during wakefulness, specifically wakefulness combined with motor activity and then fall silent during NREM and REM sleep [31,32]. Furthermore, serotonergic and noradrenergic neurons send inhibitory projection to orexin neurons which maintain inhibitory feedback responsible for the stability of orexin neuronal activity [33]. Figs. 3 and 4 clearly describe the role played by orexin as wakefulness initiator in sleep wake flip flop cycle as well as the detailed hierarchy of steps and mechanisms involved in the regulation and redirection of sleep wake homeostasis towards wakefulness *via* OX_1R and OX_2R activation.

The role of orexins in sleep wake cycle is further supported by preclinical studies where the levels of orexin-A are found to be high during the active, wakeful period and depleted to half their peak level values during sleep [34]. Also studies have revealed that injection of orexin-A or -B into the rodent brains markedly increases wakefulness *via* activating regions of the RAS [7,35–38]. The role of LC in orexenergic control of wakefulness has also been implicated. It has been stated that during orexinergic stimulation, if the LC neurons are optically inhibited, the orexin-dependent sleep to wake transitions are blocked. Conversely, when the same LC neurons are photo stimulated, concomitant activation of orexinergic neurons further increases the probability of wakefulness compared with orexin stimulation alone [39]. Interestingly, slow-wave sleep (SWS) could be induced in mice with acute inhibition of the orexinergic cells and also, inhibition of orexinergic

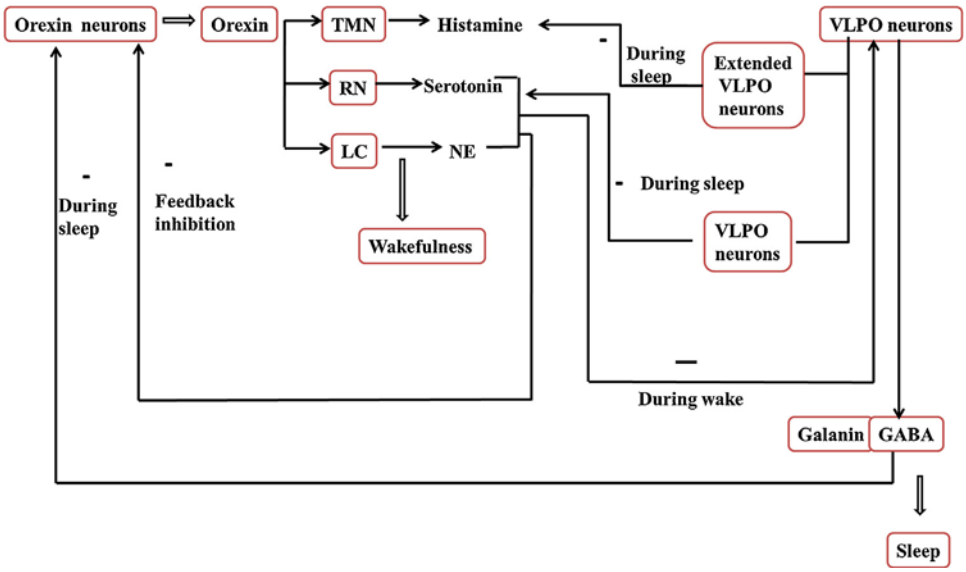

Fig. 3. Describes the initiatory transitory response of orexin signalling towards wakefulness, in the sleep wake flip flop cycle.

neurons pharmacogenetically, increases the length of SWS episodes in mice [40,41]. The involvement of orexins in the regulation of normal wakefulness is also evident from clinical and preclinical set-ups where orexin signalling was completely restricted. Orexin peptide knockout (KO) mice have severe sleepiness, with an inability to maintain long bouts of wakefulness [42]. Interestingly, these mice observe nearly normal total amounts of wakefulness, but their wake bouts are much shorter as compared to the naïve mice [43].

Switching on to details from clinical studies, narcolepsy was targeted for studying orexinergic mechanisms. It has been documented that individuals suffering from narcolepsy phenotype, roughly observe about 90% loss in the orexinergic neurons, in addition to marked reduction in CSF levels of orexin-A [9,44,45]. These observations provide compelling evidence that the orexin signalling is prime requisite for the normal maintenance and stabilisation of wakefulness.

When orexin cascade was studied in insomnia pathophysiologies specifically, it was evident that insomnia was associated with an overexpression of components of the orexinergic system. The same could be scientifically ascertained *via* exploring the changes in the expression and mutations in the orexinergic neurons as well

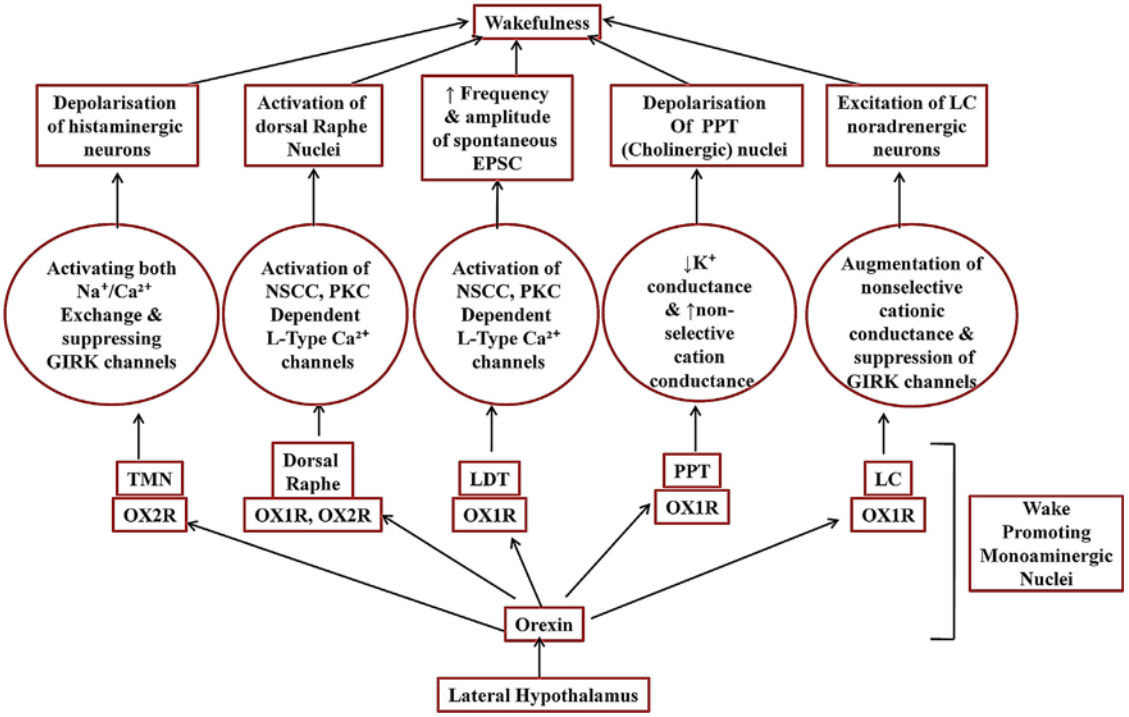

Fig. 4. Depicts the hierarchy of orexinergic receptor activation explaining the different steps/process involved in OX1R and OX_2R receptor activation and a final transition to wakefulness. It depicts the target neuronal structures for orexin activation, e.g. TMN tuberomamillary nucleus; the dorsal raphe nuclei; LDT laterodorsal tegmental nuclei; PPT pedunculopontine tegmental nuclei and the LC locus correleus, as well as the mechanisms involved in the target cells activation *via* which an initiatory wakefulness transition is accorded.

as receptors in insomniac states [46,47]. For example in the zebrafish, overexpression of orexinergic neurons has been shown to induce an insomnia-like phenotype [46]. Also research has documented that mice that overexpress prepro-orexin display sleep abnormalities which include fragmentation of nREM sleep, reduced REM sleep, and increased motor activity during REM sleep, suggesting an inability to maintain sleep states [48]. The mentioned research reports signify the potential of orexin cascade as potential target for the management of insomnia.

Mechanisms underlying the role played by orexins in maintaining wakefulness

The exact mechanism underlying the wakefulness promoting action of orexin is not well distinguished, but several hypotheses have been put forward till date. The first hypothesis proposes that orexin-1 brings about a direct effect on the release and action of almost all neurotransmitters involved in arousal-regulation. For example, studies reveal that orexin-1 microinjections into the rat's BF increases cortical acetylcholine release. Also, intracerebroventricular delivery of orexin-1 has been found to increase histamine levels in rodent frontal cortex and anterior hypothalamus [49–51]. Furthermore, microinjection of orexin-1 into other arousal modulating brain regions, like the VTA, the DR nucleus, and pontine reticular formation increases dopamine, serotonin and acetylcholine release respectively [52–54]. Concluding data from all the above mentioned studies infer that orexin might play an indirect role in intensifying the release of wakefulness promoting neurotransmitters and thus bring amplify wakefulness.

Another hypothesis recommends that orexin may increase wakefulness, *via* increasing GABA levels in the pontine reticular formation. This was evident from a study that showed that increase in wakefulness produced by microinjecting orexin into the pontine reticular formation is prevented by blocking the GABAA receptors [55].

Another alternative hypothesis represents that the primary function of orexin is to enhance activity in motor systems, while the increase in wakefulness is secondary. This hypothesis is based on the comparison of orexin concentration and firing rates of orexinergic neurons between wakefulness with or without movement. Supporting this hypothesis, research data reveals that orexin-1 concentration in the cerebrospinal fluid as well as the firing potential of the orexinergic neurons is significantly greater during active wakefulness combined with movement rather than during wakefulness alone [32,56]. Additionally even oral administration of orexin-1 and -2 receptor antagonists has also been found to increase NREM and REM sleep in rat, dog, and human as well [57]. Summarising, regardless of the plausible mechanisms, the orexins can be classified as wakefulness promoting neuropeptides.

Orexin antagonists

Insomnia has recently become a common clinical problem that has adverse impacts on individuals as well as society. 10–20% of people suffer from insomnia on a chronic basis [58]. Currently, benzodiazepine receptor agonists (BzRAs) are the most frequently prescribed drugs for treating primary insomnia. However, treatment of insomnia with BzRAs is accompanied by substantial adverse effects. Thus, the need of the hour was to research upon some alternative therapeutic targets with promising potential for the management of insomnia.

The discovery of orexin system substantially redirected the scope of pharmacotherapy for insomnia management. The fact that narcolepsy, characterised by imperative sleep attacks and excessive daytime sleepiness, is precipitated by a marked loss of orexinergic neurons, implicated that orexin plays a pivotal role in maintaining wakefulness. Thus it was thought; antagonising the orexin cascade during the night might reduce hyper-arousal, and improve sleep continuity.

During the last decade, several groups within the pharmaceutical industry have developed orexin receptor antagonists in order to identify the physiological role of orexin receptors and explore the potential of orexin receptor antagonists as therapeutic agents for management of insomnia [59]. The orexin receptor antagonists can be classified on the basis of receptor binding affinities, either as Selective Orexin Receptor Antagonists (SORAs) or Dual Orexin Receptor Antagonists (DORAs).

Selective Orexin Receptor Antagonists (SORAs)

As the name suggests, these agents bind selectively to only one of the orexin receptors (either OX1 or OX2) and thereby cause potential inhibition of the corresponding receptor functionalities. These agents were the first to be developed in the category of orexin receptor antagonists. Once it was evident from studies, that orexin receptors play pivotal role in maintaining wakefulness, the research was shifted towards deciphering the individual roles played by each of the two orexin receptors in sleep/wake homeostasis. So the development of SORAs was never aimed towards the management of insomnia but to act as probes to decipher the individual roles played by each of the orexin receptors, as to which receptor is linked to regulation of what phase and pathology of arousal. From the studies it was implicated that while arousal was primarily governed by OX_2R signalling, the simultaneous inhibition of OX_1R further attenuates the sleep-promoting effects mediated by selective OX_2R blockade. Also, it was evident that the switching between stages of sleep architecture was governed by signalling of both OX1 and OX2 receptors [60,61].

Orexin-1 receptor antagonists

These agents were developed to cause the specific inhibition of OX_1R. It has been suggested from the previous models as well, that targeting the OX_1R did not promote sleep as effectively as with OX_2R [61]. The first OX_1R antagonist developed was SB-334867 (a heterocyclic urea developed by GlaxoSmithKline, Brentford, London, United Kingdom (GSK) that bound to OX_1R and was found to promote REM sleep without any significant increase the total sleep time; however it neither decreased wakefulness nor reduced sleep latency. The activity of SB-334867 over OX_1R was found to be approximately 50-fold higher than that for OX_2R [62,63], but at higher doses, SB-334867 was likely to block both orexin receptors, complicating the interpretation of its effects [64]. Also, at higher concentrations, SB-334867 demonstrated relevant off target affinities to other CNS targets. It showed binding abilities to the monoamine and norepinephrine transporters, as well as the adenosine A3 and the 5-HT 2B receptors [61], thereby assigning a question to its pharmacological value as orexin modulators in the management of insomnia as well giving a possibility of other secondary effects pertaining to off-site receptor modulations. Furthermore, SB-334867 was reportedly degraded when stored as a solution and showed decomposition to an inactive form when stored as a hydrochloride salt [65]. These findings introduce a number of confounding influences to studies employing SB-334867 and implicate that caution should be taken in interpreting data regarding the functional roles of individual receptors based on antagonistic studies alone. Although the effect of SB-334867 was not substantial on sleep induction, this molecule has still proven to be useful for the treatment of other conditions, such as substance abuse, withdrawal, obesity and panic disorder [66–68].

Other selective OX_1R antagonists include agents like SB-408124, SB-674042 and the newest ACT 335827. But it's noteworthy that so far neither SB-408124 nor ACT-335827 has been found to promote sleep and manage insomnia. SB-408124 was shown to have almost 70 times more selectivity for OX_1R over OX_2R receptors and also exhibited improved oral bioavailability as compared to the older OX_1R antagonists like SB-334867, but the insufficient encephalic concentrations of SB-408124 due to depleted blood brain barrier crossing propensity may be held responsible for the absence of its expected positive effects [69,70].

Orexin-2 receptor antagonists

During the initial phase of research on the orexin receptor antagonists, it became evident that OX_2R plays a more crucial role in maintaining sleep wake homeostasis and thus, this receptor can be productively targeted for the treatment of insomnia. Few OX_2R receptor antagonist molecules that have been tried for their effects on sleep promotion and maintenance are EMPA, TCS-OX2-29, JNJ-10397049, MK-1064 and MK-3697 and the recent addition JNJ-42847922. Among these, EMPA (N-ethyl-2-[(6-methoxy-pyridin-3-yl)-(toluene-2-sulphonyl)-amino]-N-pyridin-3-ylmethyl-acetamide) was the first to be deciphered and developed. Studies have concluded that it had the least effective sleep promoting action amongst various SO2RA studied so far but exhibited 900-fold selectivity in binding to OX_2R over OX_1R [71]. EMPA administration has been reported to exhibit a selective increase in NREM sleep but produced no effect on REM sleep or change in sleep latency [69]. Another SO2RA developed was JNJ-10397049, which was better in activity as compared to EMPA and produced a substantial increase in total sleep time as well as an increase in both REM as well as NREM sleep [60]. JNJ-103970492 significantly decreased the latency to REM sleep while increasing the total length of each REM bout, but only at low doses of 3 mg/kg. However, at higher doses (30 mg/kg), this drug did not produce any noticeable positive effects on sleep architecture. However, further development of that molecule was stopped due to poor drug-like properties including low oral bioavailability, poor solubility and cytochrome P450 interactions. Furthermore, microdialysis assays showed that this compound reduced histamine release in the LH, therefore, it directly depleted the release of wakefulness promoting neuro-transmitters, as is conventional mechanistic approach to orexin action antagonism [61]. As the research in this dominion progressed, another OX_2R specific antagonist was developed named TCS-OX2-29. ICV administration of TCS-OX2-29 (40 nmol), increased the total sleep time by 7% in comparison to controls, and also brought about a selective increase in REM sleep in preclinical set up [72]. Most animal studies support the notion that OX_2R antagonists are much more helpful as sleep inducing agents in comparison to the OX_1R antagonists. Research database recently reported the development of some newer SO2RAs like of LSN2424100, a sulfonamide developed by Eli Lily [73]. Interestingly, this selective OX_2R antagonist displayed antidepressant-like activity in rodents [73]. Other agents include MK-1064 and MK-3697 lately disclosed by Merck and are purported to be potential clinical candidates by the developer [74,75].

The most recent addition to the SO2RA is the JNJ-42847922 which has been characterised with a dose-dependent reduction in NREM latency and increased NREM sleep duration in whereas REM sleep was minimally affected in preclinical protocols. With ongoing research and prediction of plausible human pharmacokinetic profile by utilizing *ex vivo* autoradiography as well as *in vivo* preclinical studies JNJ-42847922 was identified as a clinical candidate for the treatment of insomnia and is presently undergoing phase II trials in insomnia patients without Psychiatric Comorbidity [76].

When administered to healthy subjects, JNJ-42847922 demonstrated a consistent and rapid hypnotic-like effect with even single dose administration of 10–80 mg. It was well tolerated and exhibited favourable pharmacokinetic profile for a sedative/hypnotic, characterized by rapid absorption and short half-life. It has been recently reported that preliminary EEG polysomnography data obtained from a phase 1b study showed that treatment with JNJ-42847922 resulted in significant improvement in both sleep onset and total sleep duration in patients with comorbid insomnia related to major depressive disorder [77]. Table 1 describes most of the SORAs (both OX_1R and OX_2R antagonists) developed so far giving details about the developer, the molecule characteristics, effects observed in preclinical protocols and their present research status.

Dual Orexin Receptor Antagonists (DORAs)

With extensive research being carried over orexin receptor antagonists it became apparent that complete inhibition of orexin

Table 1
OX_1R and OX_2R receptor antagonists.

Compound	Developer	Specificity	Conclusion
EMPA (OX_2R antagonist)	Hoffmann–La Roche	OXR2 > OXR1 (900 fold)	Reduces spontaneous locomotion and blocks the increase in locomotion induced by an orexin-B fragment [60,71]
JNJ-10397049 (OX_2R antagonist)	Johnson & Johnson	OXR2 > OXR1 (630 times)	Significantly increases REM and non- REM sleep; reduces active wake [60,78]
SB-40bvg 8124 (OX_1R antagonist)	GlaxoSmithKline	OXR1 > OXR2 (70 times)	No significant effects on sleep [78]
SB -334867 (OXR1 antagonist)	GlaxoSmithKline	OXR1 > OXR2(50 fold)	First selective OXR1 antagonist. Counteract the suppression of REM sleep, does not decrease wakefulness and sleep latency, no increase in total sleep time [63,79,80]
SB -674042 (OX_1R antagonist)	GlaxoSmithKline	OXR1 > OXR2 (130 fold)	Not effective in promoting sleep [81]
ACT -335827 (OX_1R antagonist)	Actelion	OXR1 > OXR2	Orally available, penetrates the brain decreases fear, compulsive behaviours and autonomic stress reactions No substantial effects on sleep characteristics [70,82]
TCS-OX2-29 (OX2 antagonist)	Banyu	OXR2 > OXR1	First non-peptide OXR2 antagonist, decreased food intake and waSter intake [72,83]
LSN2424100	Eli Lily	OXR2 > OXR1 (200 fold)	OXR2 specific antidepressant activity [73]
MK-1064	Merck	OXR2 > OXR1	Clinical candidate. Increase in Rem sleep [74]
JNJ-42847922	Janssen	OXR2 > OXR1	Undergoing Phase II trials for insomnia. Reduces latency to (NREM) sleep and prolonged NREM sleep time. Increased somnolence in preclinical as well as phase Ib studies [77,84]
MK-3697	Merck	OXR2 > OXR1	Clinical candidate, presently in phase II, significant reductions in active wake and increases in SWS and REM sleep upon oral administration in rats and dogs [75]

precursor, i.e. prepro-orexin or loss of orexin neurons resulted in more distinct sleep induction than loss of any one of the orexin receptors. Therefore, it was hypothesized that antagonizing both orexin receptors would elicit the most powerful sleep-promoting effects [69]. Also antagonising orexin receptors individually and selectively did not bring about promising response as insomnia therapeutics. These facts provided rationale for the development of Dual Orexin Receptor Antagonists (DORAs). Studies then converted this hypothesis into an inference, such that today most of DORAs are currently undergoing clinical trials and getting FDA approval for the treatment of insomnia. Currently, the most widely debated DORA molecules in the literature are SB-649868 (a piperidine amide) developed by GSK (Brentford, London, United Kingdom), Almorexant (a tetrahydroisoquinolone) developed by Actelion (Basel, Switzerland), Suvorexant (MK-4305; a diazepane) and MK-6096 (a piperidine carboxamide), both been developed by Merck (New Jersey, USA). Table 2 describes most of the DORAs developed so far giving details about the molecule characteristics, effects observed in preclinical and clinical protocols, adverse effects profile and their present status.

Almorexant

ACT-078573 also designated as Almorexant/Restora, is chemically a tetrahydroisoquinoline. It is the most widely studied DORA and one of the first to enter phase III clinical trials (NCT00608985). Earlier preclinical studies performed on rats, dogs and mice have inferred that the administration of almorexant before lights out increased the total length of REM and NREM episodes and further reduced the wake time in a dose dependent manner [57,87]. The mechanism behind sleep-inducing effect of almorexant was proposed to be related to the specific inhibition of OX_2R rather than OX_1R. This conclusion was made from studies in KO mice, wherein upon administration of almorexant, the researchers did not observe any significant changes in the amount of sleep in the OX_2R KO mice, but sleep architecture was surely altered significantly in the OX_1R KO mice [89].

Clinical administration of almorexant has elicited effects like depleted alertness, increased fatigue, drowsiness, sleepiness, and sleep efficiency, measured as an increase in SWS and REM sleep [57]. In patients suffering from primary insomnia, its administration has proved to be effective for boosting sleep, increasing total

Table 2
Dual Orexin Receptor Antagonists (DORAs).

Compound	T1/2 (h)	Metabolism	Effects in pre-clinical trials	Effects in clinical trials	Adverse effects	Status
ACT-078573 (Almorexant) Actelion and GlaxoSmithKline	8–9	Hepatic CYP3A4 (S/I)	Low to moderate bioavailability, penetrates brain well, increases NREM and REM sleep Induce somnolence, decrease active wake, and locomotor activity, reduces muscle tone [85–87,89]	Reduces locomotor activity, increases sleep cataplectic episodes, Improves sleep efficiency, decreases sleep initiation and time spent in SWS stage [88–90]	Withdrawn by company due to issues regarding tolerability and adverse effects like headache, fatigue, blurred vision, and may even cause sleep paralysis [89]	Phase III, discontinued (2013) Subjects: 709
MK-4305 (Suvorexant) MERCK & Co	12	Hepatic CYP3A4 (S)	Reduces active wake and increases NREM and REM sleep, Decreases locomotor activity orally bioavailable, has good brain penetrance [91,92]	Effectively decreases sleep latency increases maintenance of sleep, No rebound insomnia [93–95]	It is well tolerated, but hypnagogic hallucinations and daytime sleepiness was observed as mild side effects [94]	FDA approved (2014) (Belsomora)
MK-6096 (Filorexant) Merck & Co	3–6	Hepatic CYP3A4 (S/I)	Effective in primary insomnia, migraine prophylaxis, and insomnia associated with depression [96,98]	Reduction in active wakefulness increase in slow wave sleep and prolonged sleep and a decrease in latency for both state. Binds orexin receptors more rapidly (approx. 2-fold) than almorexant [98]	No relevant side effects reported [96,98]	Phase II completed (2014) Subjects: 326
SB-649868 GlaxoSmithKline	3–6	Hepatic CYP3A4 (S/I)	Reduces latency to persistent sleep & wake after sleep onset, and increases total sleep time [100]	Improves sleep induction and sleep maintenance, Increases total sleep time and prolonged sleep time without any motor co-ordination impairment, reduces sleep latency EEG showing increase in θ, α and β waves after 2 h prior to dosing [101]	safe and well tolerated at doses up to 80 mg. the only mild side effects reported were somnolescence and fatigue [101]	Phase II completed (2012) Subjects: 48
Lemborexant Eisai Inc.	–	–	Parent compound from which it was optimized showed decrease in wakefulness as well as promotion of non-REM sleep, while REM sleep showed no significant difference [109]	Improved mean sleep efficiency as compared to placebo. It shortened both latency to persistent sleep and wake after sleep onset [110]	Somnolence, headache and sleep paralysis [110]	Phase II completed (2015). Subjects: 616
ACT-462206 Actelion Pharmaceuticals	–	–	decreases wakefulness and increases non-rapid eye movement (non-REM) and REM sleep while maintaining natural sleep architectures in rat and dog electroencephalography/ electromyography (EEG/ EMG) experiments [111]	Well tolerated, the effect on sleep was found to be dose dependent. Earlier onset of action as compared to almorexant [112]	clinically relevant reduction in vigilance and attention, alertness, and motor coordination [112]	Phase I completed (2012) Subjects: 56

sleep time, and reducing both REM sleep latency and the frequency of awakening [59]. This effect was inferred to be dose dependent with the most pronounced effect achieved at doses of 400 mg; though doses of 100 and 200 mg had modest effects on sleep with fewer adverse effects (e.g. headache, dizziness, blurred vision). Despite the fact that various studies have purported almorexant to possess promising potential for the treatment of insomnia, still the pharmaceutical company sponsoring its research, discontinued the clinical trials in 2011 citing concerns over "safety parameters" that required further evaluation. Currently, the potential of almorexant is being tested in newer alternative clinical trials for evaluation of its effect on cognitive performance (NCT01243060) [88].

Filorexant/(MK-6096)

MK-6096 represents a new series of DORAs, developed by Merck and Co., New Jersey, USA. Chemically it represents a 2,5-disubstituted piperidines with a molecular structure slightly varied from that of almorexant and suvorexant. It is an orally bioavailable, potent DORA that demonstrates binding as well as antagonism of both OX_1R and OX_2R as evidenced by radio ligand binding and functional cell based assays. The efficacy of filorexant over sleep induction has been well reported in preclinical studies where it has dose-dependently demonstrated sleep promotion effects in sleep in rats and dogs [96,98]. Preclinical administration in rats and dogs led to a significant dose-dependent decrease in active wakefulness corresponding with an increase in light and deep Slow Wave Sleep as well as depletion in sleep latencies [96,98]. In comparison to almorexant, MK-6096 has reportedly higher bioavailability as well as it induces its effects at markedly lower doses than almorexant. [96,97]. Recently, MK-6096 is under phase II clinical trials and is being investigated against episodic migraine, primary insomnia as well as diabetic neuropathy [99].

SB-649868

SB-649868 manufactured by GSK (Brentford, London, United Kingdom), is another potent orally active DORA. It is being investigated nowadays for the treatment of primary insomnia as there is piled up evidence for its effectiveness in promoting sleep, in both pre-clinical and clinical studies. Studies have inferred that administration of SB-649868 in rats corresponding to doses of 10 and 30 mg/kg brought about an increase in both NREM and REM sleep reduced sleep latency and also did not produce any motor impairment, even when the orexin antagonist was administered concurrently with ethanol [100]. In preclinical studies on rodents, the compound also depicted striking *in vivo* activity as compared to almorexant. Thus it was thought to take this compound ahead towards clinical trials [100]. In a clinical study designed by Bettica et al., it became evident that administration of SB-649868 to healthy volunteers in a noise-disturbed sleep protocol induced somnolence and fatigue at 10 and 30 mg doses [101]. Furthermore, in another clinical set up where SB-649868 was dispensed to patients diagnosed with primary insomnia it was reported that the compound produced a significant improvement in the quality of sleep (10, 30 and 60 mg) as evidenced by increase in total sleep time, depletion in sleep latency and number of night time awakenings [102]. During this clinical study, the most common adverse effects reported were headache, dry mouth and nasopharyngitis. SB-649868 has successfully passed through Phase II clinical trials (NCT00426816) [103]. Other than primary insomnia, sleep disturbances are also associated as an ancillary disorder with many primary disorders like Alzheimer's disease, Parkinson's disease, cardiac dysfunctions, etc. So the developing company of SB-649868, i.e. GSK tried to evaluate the safety and efficacy profile as well as interaction of this orexin antagonist with the first line treatment options used for above mentioned primary disorders. For example different clinical protocols have been designed and undertaken by GSK to investigate the effect of repeated doses of SB-649868 on the pharmacokinetics of simvastatin and atorvastatin, its effects on cardiac functions in healthy volunteers, as well as interaction with alprazolam and consequences produced on neuroendocrine and sympathetic responses when administered in patients with insulin induced hypoglycaemic [104].

Suvorexant

Another selective DORA developed and marketed by Merck and Co. (New Jersey, USA) is Suvorexant. It is being marketed by the name of Belsmora and is the only Dual Orexin Receptor Antagonist till date to get approval for sale by the U.S. FDA on August 13, 2014 [105]. The Drug Enforcement Organisation [DEO] of the United States has placed suvorexant on list of schedule IV controlled substances with an effective date of September 29, 2014 [106]. According to the official website, the drug would have been available in USA in early 2015 [107] as well as was set to hit the market in Japan [ahead of Pharmaceutical and Medical Devices Agency (PMDA) approval] somewhere in November 2014 [108].

Suvorexant has a demonstrated to possess a better pharmacological profile than almorexant. Preclinical studies in rats, dogs and monkeys have revealed that suvorexant in comparison to almorexant reduced active wake time by increasing NREM and REM sleep and positively modified sleep architecture at much lower doses than almorexant [87]. Also, another striking feature giving an edge to suvorexant over almorexant was that suvorexant administration was not associated with any next-day residual effects, no rebound insomnia, complex sleep-related behaviours or withdrawal effects even after 4 weeks of continuous intake [93]. Strikingly, this may be regarded as the plausible reason for the withdrawal of almorexant in clinical trials, as the high doses of almorexant required to achieve therapeutic effects could also serve as a cause of more severe adverse effects not observed in suvorexant that required doses, almost 10 times smaller.

In clinical protocols as well suvorexant in lowest doses (10 mg) administered to healthy volunteers, effectively reduced the number of awakenings after sleep onset; and at higher doses (50 mg), it reduced sleep latency while increasing total sleep time [86]. The beneficial results of Suvorexant over sleep latency and maintenance exhibited in the healthy volunteers were also reproduced in patients with primary insomnia, wherein, it successfully reduced sleep latency and increased the sleep time after just a single administration [94]. Polysomnographic study involved two 4-week protocol of oral administration of suvorexant at doses of 10, 20, 40 and 80 mg. The treatment was shown to have significant dose-related effects over sleep induction and maintenance [95]. The most frequent dose dependent adverse effects associated with suvorexant administration in the above mentioned study were mild somnolence, headaches, dizziness and abnormal dreams and that too mostly reported at higher doses [93]. As the beneficial profile of suvorexant substantially out-powered its adverse effects, this is the reason it has passed through all the stages of FDA approval and soon would establish itself as a trusted therapeutic agent for the treatment of primary insomnia.

Lemborexant

A constant interest in the orexin system from industry as well as academia over the last 17 years resulted in a vast amount of results summarized in the scientific literature and covered by numerous patent applications. The recent additions to this race are agents like Lemborexant and ACT-462206. Lemborexant (E-2006) is a dual antagonist to the OX_1R and OX_2R, under development by the developer named Eisai (Bunkyo-Ku, Japan). The parent compound

through which it was developed was exhibiting sleep promoting effects in rats at 30 mg/kg and 100 mg/kg following oral administration as depicted by dose-dependent responses like decrease in wakefulness as well as promotion of non-REM sleep, while REM sleep showed no significant difference [109]. Recently at a meeting of American College of Neuropsychopharmacology, Eisai presented the results of phase II clinical study over lemborexant and demonstrated that E2006 statistically significantly improved mean sleep efficiency as compared to placebo. It shortened both latency to persistent sleep (LPS) and wake after sleep onset (WASO). Moreover, the only E2006 group to show a statistically significant increase compared to placebo in next-day residual sleepiness as measured by the Karolinska Sleepiness Scale (KSS). However, the most common adverse events reported in patients treated with E2006 were somnolence, headache and sleep paralysis [110]. Eisai is now in terms with preparing for phase III trials for lemborexant.

Edge of DORAs over classic insomnia treatments

A number of conventional treatments are available commercially for management of insomnia and other sleep disturbances. The first FDA approved drugs for treatment of insomnia were benzodiazepines (BZDs) (estazolam, quazepam, triazolam, flurazepam and temazepam) and non BZDs, also classified as z-drugs (zaleplon, zolpidem, and eszopiclone). Lately antidepressants have also been used for the management of insomnia. Out of which tricyclic antidepressant (TCA) doxepin is the mostly widely used agent. The sleep promoting effects of doxepin are thought to be related to mainly its H1 receptor antagonism rather than its serotonin/nor epinephrine re-uptake inhibition. Melatonin agonists have also been used for the management of insomnia. Ramelteon, a melatonin agonist that acts upon MT1 and MT2 receptors improves sleep-onset latency and is approved by FDA for the management of insomnia. Despite a paucity of clinical efficacy data, antihistamines and antipsychotics are also sometimes readily available over the counter for insomnia management [113,114].

The prospective advantage of DORAs over classic insomnia treatments, such as BZDs, is the possibility of inducing more somnolent physiological sleep which is due to the fact that DORAs enhance REM sleep while BZDs subdue REM sleep [115]. Further, while the conventional treatments of insomnia are associated with major side effects like hangover, development of dependence and tolerance, rebound insomnia, muscular atonia, interaction with alcohol, cognitive disorder including amnesia, and increased anxiety during daytime or sometimes irreversible adverse effects (e.g. anticholinergic effects of tricyclic antidepressants, tardive dyskinesia related to neuroleptics), the orexin antagonists on the other hand presented a better and less intense side effects profile [115–117]. They are associated with only mild complaints of headaches and dizziness being the most common side effects. Therefore the therapeutic management of sleep disturbances needs to be redirected towards the development and use of orexin receptor antagonists for a sustained, efficient and safer management of the disorder.

Conclusion

Sleep research has now been extensively directed towards understanding the orexin system, which has provided newer insights into interpreting the neurobiology of arousal and sleep. The management of insomnia has always been a challenge as it may be influenced by a number of etiological factors and involves complex molecular and receptor mechanisms. The recent development of orexin antagonists has provided improved avenues for the management of sleep disorders; surpassing the adverse effects of conventional therapies like the benzodiazepines. Antagonism of orexin receptors have led to the development of novel therapeutic agents like SORAs and DORAs with greater pharmacological effect and fewer side effects compared to BZD and hypnotics. However, more randomized controlled trials are needed to assess both the short- and long-term effects of these medications, as well as their efficacy in comorbid diseases that affect the sleep architecture. Furthermore, orexins are involved in multiple pathways in regulating transition of sleep and wakefulness, so a clear understanding of these pathways and targeting those pathways can be further helpful to develop other novel therapeutic agents targeting treatment of insomnia.

Conflict of interest statement

The authors have no competing financial interests to declare. There is no conflict of interest between any of the authors.

Acknowledgement

Authors would like to thank the University Grants Commission (UGC), New Delhi, India for providing the financial support under the UGC-RFSMS (University Grants Commission Research Fellowship in sciences for meritorious students).

References

[1] Roth T. Diagnosis and management of insomnia. Clin Cornerstone 2005;2:28–38.
[2] Fuller PM, Gooley JJ, Saper CB. Neurobiology of the sleep-wake cycle: sleep architecture, circadian, regulation, and regulatory feedback. J Biol Rhythms 2006;21:482–93.
[3] Schwartz JR, Roth T. Neurophysiology of sleep and wakefulness basic science and clinical implications. Curr Neuropharmacol 2008;6:367–78.
[4] España RA, Baldo BA, Kelley AE, Berridge CW. Wake-promoting and sleep-suppressing actions of hypocretin (orexin): basal forebrain sites of action. Neuroscience 2011;106:699–715.
[5] Kilduff TS, Peyron C. The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. Trends Neurosci 2000;23:359–65.
[6] Sakurai T, Mieda M. Connectomics of orexin-producing neurons: interface of systems of emotion, energy homeostasis and arousal. Trends Pharmacol Sci 2011;32:451–62.
[7] de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 1998;95:322–7.
[8] Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998;92:573–85.
[9] Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000;27:469–74.
[10] Mieda M, Sakurai T. Orexin (hypocretin) receptor agonists and antagonists for treatment of sleep disorders. Rationale for development and current status. CNS Drugs 2013;2:83–90.
[11] Liu RJ, van den Pol AN, Aghajanian GK. Hypocretins(orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. J Neurosci 2002;22:9453–64.
[12] van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB. Presynaptic and postsynapticactions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. J Neurosci 1998;18:7962–71.
[13] Borgland SL, Taha SA, Sarti F, Fields HL, Bonci A. Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. Neuron 2006;49:589–601.
[14] Borgland SL, Storm E, Bonci A. Orexin B/hypocretin 2 increases glutamatergic transmission toventral tegmental area neurons. Eur J Neurosci 2002;28:1545–56.
[15] Lu XY, Bagnol D, Burke S, Akil H, Watson SJ. Differential distribution and regulation of OX1 and OX2 orexin/hypocretin receptor messenger RNA in the brain upon fasting. Horm Behav 2000;37:335–44.
[16] Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, et al. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 2001;435:6–25.
[17] Gvilia I, Xu F, Mc Ginty D, Szymusiak R. Homeostatic regulation of sleep: a role for preoptic area neurons. J Neurosci 2006;26:9426–33.
[18] Sherin JE, Elmquist JK, Torrealba F, Saper CB. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. J Neurosci 1998;18:4705–21.

[19] Lu J, Bjorkum AA, Xu M, Gaus SE, Shiromani PJ, Saper CB. Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. J Neurosci 2002;22:4568–76.
[20] Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, et al. Identification of sleep-promoting neurons in vitro. Nature 2000;404:992–5.
[21] Sakurai T, Mieda M, Tsujino N. The orexin system: roles in sleep/wake regulation. Ann N Y Acad Sci 2010;1200:149–61.
[22] Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, et al. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron 2003;38:701–13.
[23] Xie X, Crowder TL, Yamanaka A, Morairty SR, Lewinter RD, Sakurai T, et al. GABA(B) receptor-mediated modulation of hypocretin/orexin neurones in mouse hypothalamus. J Physiol 2006;574:399–414.
[24] Lee MG, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J Neurosci 2005;25:6716–20.
[25] Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, et al. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci U S A 1999;96:10911–16.
[26] Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, et al. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. Brain Res 2000;873:181–7.
[27] Brown RE, Sergeeva OA, Eriksson KS, Haas HL. Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems (orexin/hypocretin, histamine and noradrenaline). J Neurosci 2002;22:8850–9.
[28] Yamanaka A, Tsujino N, Funahashi H, Honda K, Guan JL, Wang QP, et al. Orexins activate histaminergic neurons via the orexin 2 receptor. Biochem Biophys Res Commun 2002;290:1237–45.
[29] Eggermann E, Serafin M, Bayer L, Machard D, Saint-Mleux B, Jones BE, et al. Orexins/hypocretins excite basal forebrain cholinergic neurones. Neuroscience 2001;108:177–81.
[30] Alam MN, Szymusiak R, Gong H, King J, McGinty D. Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. J Physiol 1999;521:679–90.
[31] Lee MG, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J Neurosci 2005;25:6716–20.
[32] Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 2005;46:787–98.
[33] Yamanaka A, Muraki Y, Ichiki K, Tsujino N, Kilduff TS, Goto K, et al. Orexin neurons are directly and indirectly regulated by catecholamines in a complex manner. J Neurophysiol 2006;96:284–98.
[34] Yoshida Y, Fujiki N, Nakajima T, Ripley B, Matsumura H. 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 2001;14:1075–81.
[35] Piper DC, Upton N, Smith MI, Hunter AJ. The novel brain neuropeptide, orexin-A, modulates the sleep-wake cycle of rats. Eur J Neurosci 2000;12:726–30.
[36] Kohlmeier KA, Watanabe S, Tyler CJ, Burlet S, Leonard CS. Dual orexin actions on dorsal raphe and laterodorsal tegmentum neurons: noisy cation current activation and selective enhancement of Ca^{2+} transients mediated by L-type calcium channels. J Neurophysiol 2008;100:2265–81.
[37] Bayer L, Serafin M, Eggermann E, Saint-Mleux B, Machard D, Jones BE, et al. Exclusive postsynaptic action of hypocretin-orexin on sub layer 6b cortical neurons. J Neurosci 2004;24:6760–4.
[38] Eriksson KS, Sergeeva O, Brown RE, Haas HL. Orexin/hypocretin excites the histaminergicneurons of the tuberomammillary nucleus. J Neurosci 2001;21:9273–9.
[39] Carter ME, Brill J, Bonnavion P, Huguenard JR, Huerta R, deLecea L. Mechanism for hypocretin-mediated sleep-to-wake transitions. Proc Natl Acad Sci USA 2012;109:E2635–44.
[40] Tsunematsu T, Kilduff TS, Boyden ES, Takahashi S, Tominaga M, Yamanaka A. Acute optogenetic silencing of orexin/hypocretin neurons induces slow-wave sleep in mice. J Neurosci 2011;31:10529–39.
[41] Sasaki K, Suzuki M, Mieda M, Tsujino N, Roth B, Sakurai T. Pharmacogenetic modulation of orexin neurons alters sleep/wakefulness states in mice. PLoS ONE 2011;6:e20360.
[42] Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE. Behavioral state instability in orexin knock-out mice. J Neurosci 2004;24:6291–300.
[43] DinizBehn CG, Klerman EB, Mochizuki T, Lin SC, Scammell TE. Abnormal sleep/wake dynamics in orexin knockout mice. Sleep 2010;33:297–306.
[44] Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2005;6:991–7.
[45] Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002;59:1553–62.
[46] Prober DA, Rihel J, Onah AA, Sung RJ, Schier AF. Hypocretin/orexin over expression induces an insomnia-like phenotype in zebrafish. J Neurosci 2006;26:13400–10.
[47] Yokogawa T, Marin W, Faraco J, Pézeron G, Appelbaum L, Zhang J, et al. Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. PLoS Biol 2007;5:e277.
[48] Willie JT, Takahira H, Shibahara M, Hara J, Nomiyama M, Yanagisawa M, et al. Ectopic overexpression of orexin alters sleep/wakefulness states and muscle tone regulation during REM sleep in mice. J Mol Neurosci 2011;43:155–61.
[49] Dong HL, Fukuda S, Murata E. Orexins increase cortical acetylcholine release and electroencephalographic activation through orexin-1 receptor in the rat basal forebrain during isoflurane anesthesia. Anesthesiology 2006;104:1023–32.
[50] Hong ZY, Huang ZL, Qu WM. Orexin A promotes histamine, but not norepinephrine or serotonin, release in frontal cortex of mice. Acta Pharmacol Sin 2005;26:155–9.
[51] Ishizuka T, Yamamoto Y, Yamatodani A. The effect of orexin-A and -B on the histamine release in the anterior hypothalamus in rats. Neurosci Lett 2002;323:93–6.
[52] Vittoz NM, Berridge CW. Hypocretin/orexin selectively increases dopamine efflux within the prefrontal cortex: involvement of the ventral tegmental area. Neuropsychopharmacol 2006;31:384–95.
[53] Tao R, Ma Z, Mc Khenna JT, Thakkar MM, Winston S, Strecker RE, et al. Differential effect of orexins (hypocretins) on serotonin release in the dorsal and median raphé nuclei of freely behaving rats. Neuroscience 2006;141:1101–5.
[54] Bernard R, Lydic R, Baghdoyan HA. Hypocretin (orexin) receptor subtypes differentially enhance acetylcholine release and activate G protein subtypes in rat pontine reticular formation. J Pharmacol Exp Ther 2006;317:163–71.
[55] Brevig HN, Watson CJ, Lydic R, Baghdoyan HA. Hypocretin GABA interacting the pontine reticular formation increase wakefulness. Sleep 2010;33:1285–93.
[56] Kiyashchenko LI, Mileykovskiy BY, Maidment N, Lam AH, Ming-Fung Wu MF, John J, et al. Release of hypocretin (orexin) during waking and sleep states. J Neurosci 2002;22:5282–6.
[57] Brisbare-Roch C, Dingemanse J, Koberstein R, Hoever P, Aissaoui H, Flores S, et al. Promotion of sleep by targeting the orexin system in rats, dogs, and humans. Nat Med 2007;13:150–5.
[58] Ohayon MM. Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev 2002;6:97–111.
[59] Hoever P, Haas SD, Winkler J, Schoemaker RC, Chiossi E, Gerven JV, et al. Orexin receptor antagonism, a new sleep-promoting paradigm: an ascending single-dose study with almorexant. Clin Pharmacol Ther 2010;87:593–600.
[60] Gozzi A, Turrini G, Piccoli L, Massagrande M, Amantini D, Antolini M, et al. Functional magnetic resonance imaging reveals different neural substrates for the effects of orexin-1 and orexin-2 receptor antagonists. PLoS ONE 2011;6:e16406.
[61] Gotter AL, Webber AL, Coleman PJ, Renger JJ, Winrow CJ. International Union of Basic and Clinical Pharmacology LXXXVI Orexin receptor function, nomenclature and pharmacology. Pharmacol Rev 2012;64:389–420.
[62] Haynes AC, Jackson B, Chapman H, Tadayyon M, Johns A, Porter RA, et al. A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. Regul Pept 2000;96:45–51.
[63] Porter RA, Chan WN, Coulton S, Johns A, Hadley MS, Widdowson K, et al. 1,3-Biarylureas as selective non-peptide antagonists of the orexin-1 receptor. Bioorg Med Chem Lett 2001;11:1907–10.
[64] Winrow CJ, Gotter AL, Coleman PJ, Hargreaves R, Renger J. Recent chronology of orexin pharmacology and its potential as a treatment for primary insomni. In: Rankovic Z, Bingham M, Nestler EJ, Hargreaves R, editors. Drug discovery for psychiatric disorders. London: The Royal Society of Chemistry; 2012. p. 416–42.
[65] McElhinny Jr CJ, Lewin AH, Mascarella SW, Runyon S, Brieaddy L, Carroll FI. Hydrolytic instability of the important orexin 1 receptor antagonist SB-334867: possible confounding effects on in vivo and in vitro studies. Bioorg Med Chem Lett 2012;22:6661–4.
[66] White CL, Ishii Y, Mendoza T, Upton N, Stasi LP, Bray GA, et al. Effect of a selective OX1R antagonist on food intake and body weight in two strains of rats that differ in susceptibility to dietary-induced obesity. Peptides 2005;26:2331–8.
[67] Johnson PL, Truitt W, Fitz SD, Minick PE, Dietrich A, Sanghani S, et al. A key role for orexin in panic anxiety. Nat Med 2010;16:111–5.
[68] Smith RJ, Aston-Jones G. Orexin/hypocretin 1 receptor antagonist reduces heroin self-administration and cue-induced heroin seeking. Eur J Neurosci 2012;35:798–804.
[69] Morairty SR, Revel FG, Malherbe P, Moreau JL, Valladao D, Wettstein JG, et al. Dual hypocretin receptor antagonism is more effective for sleep promotion than antagonism of either receptor alone. PLoS ONE 2012;7:e39131.
[70] Steiner MA, Gatfield J, Roch BC, Dietrich H, Treiber A, Jenck F, et al. Discovery and characterization of ACT-335827, an orally available, brain penetrant orexin receptor type 1 selective antagonist. Chem Med Chem 2013;8:898–903.
[71] Malherbe P, Borroni E, Gobbi L, Knust H, Nettekoven M, Pinard E, et al. Biochemical and behavioural characterization of EMPA, a novel high-affinity, selective antagonist for the OX(2) receptor. Br J Pharmacol 2009;156:1326–41.
[72] Kummangal BA, Kumar D, Mallick HN. Intracerebro ventricular injection of orexin-2 receptor antagonist promotes REM sleep. Behav Brain Res 2013;237:59–62.
[73] Fitch TE, Benvenga MJ, Jesudason CD, Zink C, Vandergriff AB, Menezes M, et al. LSN2424100: a novel, potent orexin-2 receptor antagonist with selectivity over orexin-1 receptors and activity in an animal model predictive of antidepressant-like efficacy. Front Neurosci 2014;2014(8):5.
[74] Roecker AJ, Mercer SP, Schreier JD, Cox CD, Fraley ME, Steen JT, et al. Discovery of 5-chloro-N-[(5,6-dimethoxypyridin-2-yl)methyl]-2,2′:5′,3-terpyridine-3′-carbo xamide (MK-1064): a selective orexin 2 receptor antagonist (2-SORA) for the treatment of insomnia. Chem Med Chem 2014;9:311–22.
[75] Roecker AJ, Reger TS, Mattern MC, Mercer SP, Bergman JM, Schreier JD, et al. Discovery of MK-3697: a selective orexin 2 receptor antagonist (2-SORA) for the treatment of insomnia. Bioorg Med Chem Lett 2014;24:4884–90.

[76] https://clinicaltrials.gov/ct2/show/NCT01995838 [accessed 28.08.15].
[77] http://ir.minervaneurosciences.com/JNJ-42847922 [accessed 28.08.15].
[78] Dugovic C, Shelton JE, Aluisio LE, Fraser IC, Jiang XH, Sutton SW. Blockade of orexin-1 receptors attenuates orexin-2 receptor antagonism-induced sleep promotion in the rat. J Pharmacol Exp Ther 2009;330:142–51.
[79] Smart D, Sabido-David C, Brough SJ, Jewitt F, Johns A, Porter RA. SB-334867-A: the first selective orexin-1 receptor antagonist. Br J Pharmacol 2001;132: 1179–82.
[80] Rodgers RJ, Halford JC, Nunes de Souza RL, Canto de Souza AL, Piper DC, Arch JR, et al. SB-334867, selective orexin-1 receptor antagonist, enhances behavioural satiety and blocks the hyperphagic effect of orexin-A in rats. Eur J Neurosci 2001;13:1444–52.
[81] Langmead CJ, Jerman JC, Brough SJ, Scott C, Porter RA, Herdon HJ. Characterisation of the binding of [3H]-SB-6740421, a novel non peptide antagonist, to the human orexin-1 receptor. Br J Pharmacol 2004;141:340–6.
[82] Steiner MA, Sciarretta C, Pasquali A, Jenck F. The selective orexin receptor 1 antagonist ACT-335827 in a rat model of diet-induced obesity associated with metabolic syndrome. Front Pharmacol 2013;4:165.
[83] Hirose M, Egashira S, Goto Y, Hashihayata T, Ohtake N, Iwaasa H, et al. N-acyl 6,7-dimethoxy-1,2,3,4-tetrahydroiso-quinoline: the first orexin-2 receptor selective non-peptidic antagonist. Bioorg Med Chem Lett 2003;13:4497–9.
[84] Bonaventure P, Shelton J, Yun S, Nepomuceno D, Sutton S, Aluisio L, et al. Characterization of JNJ-42847922, a selective orexin-2 receptor antagonist, as a clinical candidate for the treatment of insomnia. J Pharmacol Exp Ther 2015. http://dx.doi.org/10.1124/jpet.115.225466.
[85] Neubauer DN. Almorexant a dual orexin receptor antagonist for the treatment of insomnia. Curr Opin Investig Drugs 2010;11:101–10.
[86] Malherbe P, Borroni E, Pinard E, Wettstein JG, Knoflach F. Biochemical and electrophysiological characterization of almorexant, a dual orexin 1 receptor (OX1)/orexin 2 receptor (OX2) antagonist: comparison with selective OX1 and OX2 antagonists. Mol Pharmacol 2009;76:618–31.
[87] Pałasz A, Lapray D, Peyron C, Gołębiewska ER, Skowronek R, Markowski G, et al. Dual orexin receptor antagonists – promising agents in the treatment of sleep disorders. Int J of Neuropsychopharmacol 2014;17:157–68.
[88] https://clinicaltrials.gov/ct2/show/NCT01243060 [accessed 15.03.15].
[89] Mang GM, Durst T, Burki H, Imobersteg S, Abramowski D, Schuepbach E, et al. The dual orexin receptor antagonist almorexant induces sleep and decreases orexin-induced locomotion by blocking orexin 2 receptors. Sleep 2012;35: 1625–35.
[90] Hoever P, de Haas SL, Dorffner G, Chiossi E, van Gerven JM, Dingemanse J. Orexin receptor antagonism: an ascending multiple-dose study with almorexant. J Psychopharmacol 2012;26:1071–80.
[91] Winrow CJ, Gotter AL, Cox CD, Doran SM, Tannenbaum PL, Breslin MJ, et al. Promotion of sleep by suvorexant-a novel dual orexin receptor antagonist. J Neurogenet 2011;25:52–61.
[92] Cox CD, Breslin MJ, Whitman DB, Schreier JD, McGaughey GB, Bogusky MJ, 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-methy l-2-(2H-1,2,3-triazol-2-yl) phenyl]methanone (MK-4305) for the treatment of insomnia. J Med Chem 2010;53:5320–32.
[93] Bennett T, Bray D, Neville MW. Suvorexant, a dual orexin receptor antagonist for the management of insomnia. PT 2014;39(4):264–6.
[94] Herring WJ, Snyder E, Budd K, Hutzelmann J, Snavely D, Liu K, et al. Orexin receptor antagonism for treatment of insomnia: a randomized clinical trial of suvorexant. Neurology 2012;79:2265–74.
[95] Sun H, Kennedy WP, Wilbraham D, Lewis N, Calder N, Li X. Effects of suvorexant, an orexin receptor antagonist, on sleep parameters as measured by polysomnography in healthy men. Sleep 2013;36:259–67.
[96] Winrow CJ, Gotter AL, Cox CD, Tannenbaum PL, Garson SL, Doran SM, et al. Pharmacological characterization of MK-6096: A dual orexin receptor antagonist for insomnia. Neuropharmacology 2012;62:978–87.
[97] Winrow CJ, Gotter AL, Cox CD, Tannenbaum PL, Garson SL, Doran SM, et al. Pharmacological characterization of MK-6096 – a dual orexin receptor antagonist for insomnia. Neuropharmacology 2012;62:978–87.
[98] Coleman PJ, Schreier JD, Cox CD, Breslin MJ, Whitman DB, Bogusky MJ, 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. Chem Med Chem 2012;7:415–24.
[99] https://www.clinicaltrialsregister.eu/ctr-search [accessed 15.03.15].
[100] Di Fabio R, Pellacani A, Faedo S, Roth A, Piccoli L, Gerrard P. Discovery process and pharmacological characterization of a novel dual orexin1 and orexin 2 receptor antagonist useful for treatment of sleep disorders. Bio org Med Chem Lett 2011;21:5562–7.
[101] Bettica P, Squassante L, Zamuner S, Nucci G, Danker HH, Ratti E. The orexin antagonist SB-649868 promotes and maintains sleep in men with primary insomnia. Sleep 2012;35:1097–104.
[102] Bettica P, Squassante L, Zamuner S, Nucci G, Danker-Hopfe H, Ratti E. The orexin antagonist SB-649868 promotes and maintains sleep in men with primary insomnia. Sleep 2012;35:1097–104.
[103] https://clinicaltrials.gov/ct2/show/NCT00426816 [accessed 15.03.15].
[104] http://www.gsk-clinicalstudyregister.com/compounds/sb649868 [accessed 20.03.15].
[105] http://fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm409950.html [accessed 17.03.15].
[106] https://federalregister.gov/articles/2014/02/13/2014-03124/schedules-of-controlled-substances-placement-of-suvorexant-into-schedule-iv [accessed 28.03.15].
[107] http://belsomra.com/ [accessed 17.03.15].
[108] http://en.apdnews.com/news/7ff7b8a890574d1cbb50675732d6873a.html [accessed 18.03.15].
[109] Yoshida Y, Taro T, Yoshimitsu N, Yuji K, Fumihiro O, Carsten T, et al. Design, synthesis, and structure–activity relationships of a series of novel N-aryl-2-phenylcyclopropanecarboxamide that are potent and orally active orexin receptor antagonists. Bioorg Med Chem 2014;22:6071–88.
[110] http://www.eisai.com/news/news201477 [accessed 28.08.15].
[111] Boss C. Orexin receptor antagonists-a patent review (2010 to August 2014). Expert Opin Ther Pat 2014;24:1367–81.
[112] Hoch M, Helene van G, Joop van G, Jasper D. Entry-into-humans study with ACT-462206, a novel dual orexin receptor antagonist, comparing its pharmacodynamics with almorexant. J Clin Pharma 2014;54:979–86.
[113] Risberg A, Risberg J, Ingvar D. Effects of promethazine on nocturnal sleep in normal man. Psychopharmacologia 1975;43:279–84.
[114] Wilson SJ, Nutt DJ, Alford C, Argyropoulos SV, Baldwin DS, Bateson AN, et al. British Association for Psychopharmacology consensus statement on evidence-based treatment of insomnia, parasomnias and circadian rhythm disorders. J Psychopharmacol 2010;24:1577–601.
[115] Lanoir J, Killam EK. Alteration in the sleep-wakefulness patterns by benzodiazepines in the cat. Electroencephalogr Clin Neurophysiol 1968;25: 530–42.
[116] Borbély AA, Mattmann P, Loepfe M, Strauch I, Lehmann D. Effect of benzodiazepine hypnotics on all-night sleep EEG spectra. Hum Neurobiol 1985;4: 189–94.
[117] Gaillard JM, Schulz P, Tissot R. Effects of three Benzodiazepines (Nitrazepam, Flunitrazepam and Bromazepam) on sleep of normal subjects, studied with an automatic sleep scoring system. Pharmacopsychiatry 2009;6:207–17.