Orexin (Hypocretin) receptor agonists and antagonists for treatment of sleep disorders: Rationale for development and current status

CNS Drugs 2013; 27 (2)

Leading Article

Running title: Orexin (Hypocretin) Receptor Agonists and Antagonists for Treatment of Sleep Disorders

Orexin (Hypocretin) Receptor Agonists and Antagonists for Treatment of Sleep Disorders

Rationale for Development and Current Status

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Abstract

Orexin A and orexin B are hypothalamic neuropeptides initially identified as endogenous ligands for two orphan G-protein coupled receptors (GPCRs). They play critical roles in the maintenance of wakefulness by regulating function of monoaminergic and cholinergic neurons that are implicated in the regulation of wakefulness. Loss of orexin neurons in humans is associated with narcolepsy, a sleep disorder characterized by excessive daytime sleepiness and cataplexy, further suggesting the particular importance of orexin in the maintenance of the wakefulness state. These findings have encouraged pharmaceutical companies to develop drugs targeting orexin receptors as novel medications of sleep disorders, such as narcolepsy and insomnia. Indeed, phase III clinical trials have been completed last year of suvorexant, a non-selective (dual) antagonist for orexin receptors, for the treatment of primary insomnia, and demonstrate promising results. The New Drug Application (NDA) for suvorexant has been submitted to the US FDA. Thus, the discovery of a critical role played by the orexin system in the regulation of sleep/wakefulness has opened the door of a new era for sleep medicine.

1 Introduction

Recent studies have established that the orexin (hypocretin) system is a critical regulator of sleep/wake states and that its deficiency results in the sleep disorder narcolepsy in humans[1]. These findings have brought about the possibility of novel therapies targeting the orexin system for sleep disorders, such as narcolepsy and insomnia. In this article, we will first review the evidence that the orexin system is involved in the control of sleep/wakefulness states and how dysfunction of the system is involved in the development of sleep disorders, such as narcolepsy and insomnia. Then, we will briefly go through the potential of orexin receptor agonists for treating disorders characterized by excessive sleepiness, including narcolepsy. We will then

discuss the antagonists of orexin receptors that are in development for the treatment of sleep disorders, and their likely future place in therapy.

2 Discovery of Orexin Peptides

Orexin A and orexin B were initially identified as endogenous ligands for two orphan G-protein coupled receptors (GPCR)[2]: GPCRs for which endogenous ligands are unknown are referred to as ‘orphan’ GPCRs. Molecular cloning studies showed that both orexin A and orexin B are derived from a common single precursor peptide, prepro-orexin[2]. Since intracerebroventricular (ICV) injection of these peptides into rats acutely stimulated food consumption, they were named orexin A and orexin B after the Greek word ‘orexis’, meaning appetite[2]. A messenger RNA (mRNA) encoding the same precursor peptide of hypocretin 1 (corresponding to orexin A) and hypocretin 2 (orexin B) was independently identified by de Lecea et al. as a hypothalamus-specific transcript[3]. Orexin A and orexin B constitute a novel distinct peptide family. Orexin A is a 33-amino acid peptide with an N-terminal pyroglutamyl residue, two intrachain disulphide bonds and C-terminal amidation, while orexin B is a 28-amino acid, C-terminally amidated linear peptide. The C-terminal half of orexin B is very similar to that of orexin A, whereas the N-terminal half is more variable.

The actions of orexins are mediated by two receptors, named orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R) [also known as HCRTR1 and HCRTR2]. OX1R shows higher affinity for orexin A than orexin B by one-order, while OX2R binds orexin A and orexin B with similar affinities[2]. Both receptors are G_q-coupled and caused strong excitatory effects on neurons examined thus far[1].

Neurons expressing orexins (orexin neurons) are distributed within the perifornical lateral hypothalamus and send projections throughout the brain and spinal cord[2-7]. The number

of these neurons has been estimated as around 3,000 ~ 4,000 in rat brains and 70,000 in human brains[8, 9]. Consistent with the broad projections of orexin neurons, OX1R and OX2R show partly overlapping but distinct distributions of their mRNA throughout the CNS[10, 11].

3 Implication of Orexins in the Pathophysiology of Narcolepsy

Human narcolepsy is a debilitating neurological disease that affects approximately 1 in 2,000 individuals in the USA[1, 12, 13]. Onset of the condition usually occurs during adolescence. Narcolepsy is characterized by excessive daytime sleepiness that often results in 'sleep attacks' (sudden onset of non-rapid eye movement [REM] sleep), cataplexy (sudden bilateral skeletal muscle weakening triggered by emotions without impairment of consciousness), hypnagogic hallucinations and sleep paralysis. These symptoms can be divided into two independent pathological phenomena. One is the inability to maintain consolidated wakefulness and sleep episodes (i.e. dysregulation of transitions between wakefulness and non-REM sleep). This phenomenon manifests clinically as excessive daytime sleepiness (or sleep attacks) and disrupted night-time sleep. The other key phenomenon is the pathological intrusion of REM sleep into wakefulness or at sleep onset (i.e. dysregulation of REM sleep onset). It is during these periods that patients may experience cataplexy, hypnagogic hallucinations and sleep paralysis.

The first indications that orexins are involved in narcolepsy came from two independent studies utilizing dog forward genetics and mouse reverse genetics. Orexin knockout mice display symptoms strikingly similar to human narcolepsy, including markedly decreased duration of wakefulness episodes during the dark phase (i.e. inability to maintain a long awake period, or sleepiness), and abrupt behavioural arrests with muscle atonia (i.e. potentially cataplexy)[14]. Orexin neuron-ablated mice and OX1R/OX2R double-deficient mice

also exhibit similar phenotypes[15, 16]. In addition, mutations in the *OX2R* gene have been demonstrated to be responsible for an inherited canine model of narcolepsy[17]. This canine model also displays emotionally triggered cataplexy, fragmented sleep patterns and excessive daytime sleepiness. These studies in animals established that genetic disruption of the orexin system, especially that of the OX2R-mediated pathway, causes narcolepsy.

Subsequently, disruptions of the orexin system in human narcolepsy were confirmed. In contrast to normal control individuals, approximately 90 % of patients with narcolepsy with cataplexy have low or undetectable levels of orexin neuropeptides in the cerebrospinal fluid (CSF)[18]. Drastic reductions of orexin mRNA and immunoreactivity in postmortem brains of narcoleptic patients were also shown[9, 19]. A recent finding showing concomitant loss of dynorphin, neuronal activity-regulated pentraxin and orexin, which all co-localize in orexin neurons, strongly indicates a selective loss of orexin neurons in narcolepsy[20]. Taken in conjunction with a strong association of human narcolepsy with certain human leukocyte antigen (HLA) alleles[21], narcolepsy is likely to be caused by a selective autoimmune degeneration of orexin neurons. An increasing number of patients with a milder form of typical narcolepsy, with daytime sleepiness and sleep-onset REM periods but without cataplexy, are being recognized[13]. In contrast to narrowly defined narcolepsy by the presence of cataplexy, most (>75 %) people diagnosed with narcolepsy without cataplexy have normal CSF orexin-A levels[18, 22].

4 Regulation of Sleep/Wakefulness by Orexin Peptides

Sleep and wakefulness are controlled by a complex network of neurotransmitters and neuromodulators, such as glutamate, GABA, glycine, serotonin, dopamine, noradrenaline (norepinephrine), histamine, acetylcholine, adenosine, neuropeptides, cytokines and

prostaglandins[23, 24]. Monoaminergic neurons, including locus coeruleus (LC) noradrenergic, dorsal and median raphe serotonergic, and tubelomammilary nucleus (TMN) histaminergic neurons, project diffusely to the cerebral cortex, thalamus and brainstem, and are thought to promote arousal. They are highly active during wakefulness, while they reduce their activities during non-REM sleep and almost cease discharge during REM sleep. In contrast, GABA/galaninergic neurons in the ventrolateral preoptic nucleus (VLPO) of the hypothalamus are active during sleep, especially during non-REM sleep, and are considered as a sleep centre. VLPO neurons and monoaminergic neurons reciprocally inhibit each other.

Orexin neurons send their projections densely to nuclei involved in sleep/wakefulness regulation, including the LC noradrenergic, raphe serotonergic, TMN histaminergic, pedunculopontine tegmental nucleus (PPT)/laterodorsal tegmental nucleus (LDT) and basal forebrain (BF) cholinergic neurons[6, 14, 25, 26]. In accordance with the innervation, neurons in these nuclei express OX1R and/or OX2R in different combinations[10, 11]. ICV administration of orexin-A in rodents reduces REM and non-REM sleep, and increases wakefulness[11, 27]. Furthermore, optogenetic excitation of orexin neurons results in increases in the probability of an awakening event during both non-REM and REM sleep[28]. Application of orexin A directly into the LC[29], TMN[30], BF cholinergic area[31] and LDT[32] has also been reported to increase wakefulness. In vitro slice electrophysiology studies have shown that orexin A and orexin B increase firing rates of monoaminergic neurons in the LC[25, 33], dorsal raphe[34, 35], TMN[26, 36, 37] and cholinergic neurons in the BF and LDT[38, 39]. These observations suggest that orexin neurons stabilize wakefulness by regulating these monoaminergic and cholinergic neurons. In addition, orexin neurons activate themselves directly and indirectly via local glutamatergic neurons, forming positive-feedback circuits that may stabilize the activity of the orexin neuron network[40, 41].

Considering symptoms of narcolepsy, orexin neurons are expected to be active during wakefulness and to be silent during sleep, as observed in wake-active monoaminergic neurons. In vivo single-unit recordings have confirmed such a wake-active firing pattern of orexin neurons[42-44].

Orexin neurons have been demonstrated to have unorthodox synaptic input organization[45]. They are controlled primarily by excitatory synaptic inputs, which outnumber inhibitory synapses by a ratio of 10 : 1. This organization is in sharp contrast to the fact that neuronal cell bodies in the CNS are either dominated by inhibitory inputs (long-projective neurons), or have an approximate ratio of excitatory to inhibitory inputs of 1 : 1. Such a unique input organization of orexin neurons may be a necessary element for the maintenance of a low threshold for arousal and alertness. On the other hand, this circuitry may also be an underlying cause of insomnia.

5 Clinical Potential of Drugs Targeting Orexin Receptors

5.1 Orexin Receptor Agonists

Because narcolepsy is a condition of orexin deficiency, replacement therapy using orexin receptor agonists could be valuable for treating narcolepsy. These drugs might also be effective in other conditions of excessive daytime sleepiness. Currently, excessive sleepiness is treated using psychostimulants, while cataplexy is treated with antidepressants[13]. To consolidate nocturnal sleep, improve daytime alertness and to reduce cataplexy, γ-hydroxybutyrate (sodium oxybate, GHB) is also used[13]. However, these therapeutic regimens are problematic due to limited effectiveness, undesirable side effects such as insomnia or symptom rebound, and the potential for drug abuse[13]. Interestingly, amphetamine abuse is rare in narcoleptic patients with

cataplexy[13], which seems relevant to the fact that orexin signalling potentiates the mesolimbic dopamine pathway[1].

Indications that treatment of narcolepsy with orexin agonists would be effective come from a study demonstrating that chronic overproduction of orexin peptides from an ectopically expressed transgene prevented the development of a narcolepsy syndrome in orexin neuron-ablated mice[46]. Acute ICV administration of orexin A also maintained wakefulness, suppressed sleep and inhibited cataplectic attacks in orexin neuron-ablated mice[46]. These findings provide strong evidence of the specific causal relationship between the absence of orexin peptides and the development of the narcolepsy syndrome. They further indicate that even after substantial periods of orexin deficiency, the neural mechanisms required for orexin-mediated arousal and suppression of cataplexy remain intact. Nevertheless, development of small-molecule orexin agonists has not yet been reported.

5.2 Orexin Receptor Antagonists

Since orexins have been implicated in the maintenance of arousal, orexin receptor antagonists have been drawing attention in the medical field as potential new drugs for treating primary insomnia[1, 47]. Some pharmaceutical companies have developed several orexin receptor antagonists with different pharmacological characteristics, and some of them have been entered into late phases of clinical trials (Table 1).

Insomnia is a common clinical problem that has numerous impacts on individuals and society: 10–20 % of people have chronic insomnia, i.e. persistent trouble sleeping more than three nights each week[47]. People with insomnia often have difficulty initiating or maintaining sleep, leading to daytime fatigue, inattention and poor mood. As a result, they have reduced productivity, higher rates of missing work and increased risk of depression or substance

abuse. Insomnia is common in people with psychiatric disorders including depression, anxiety, dementia and substance abuse, as well as disorders that disrupt sleep such as pain, sleep apnoea, restless legs syndrome and circadian rhythm disorders. In some cases, however, the insomnia has no obvious medical or psychiatric cause and is considered primary insomnia.

Currently, benzodiazepine receptor agonists (BzRAs) are the most frequently prescribed drugs for treating primary insomnia[47]. BzRAs are positive allosteric modulators of $GABA_A$ receptors and widely inhibit neuronal activity. They include traditional benzodiazepines (e.g. lorazepam, diazepam) and non-benzodiazepines (e.g. zolpidem, zaleplon). Classical benzodiazepines non-selectively bind to $GABA_A$ receptors containing a $\gamma 2$ subunit, whereas non-benzodiazepine hypnotics bind with higher relative affinity to $\alpha 1$-containing receptors. BzRAs often hasten the onset of sleep, reduce the frequency of arousal during sleep and increase the total amount of sleep. However, since GABA is the primary inhibitory neurotransmitter in the brain, involved in the regulations of every aspect of the brain's functions, treatment of insomnia with BzRAs is often accompanied by adverse effects, such as next-day residual sedation, amnestic effects, rebound insomnia, and abuse and physical dependence. Thus, it is preferred to use BzRAs for the short-term treatment of insomnia, taken intermittently and at the lowest effective dose.

In contrast to GABA, orexins are dedicated mainly to the promotion and maintenance of wakefulness. Thus, orexin antagonism may provide a novel strategy for the treatment of insomnia with fewer adverse effects. The first proof-of-concept of this strategy was demonstrated with almorexant (ACT-078573, Actelion Pharmaceuticals Ltd**,** Allschwil, Switzerland) [Table 1][48]. Almorexant blocks both OX1R and OX2R with almost equimolar potency (dual orexin receptor antagonist, DORA), is bioavailable with oral dosing and is highly selective to orexin receptors[48]. It reduces time spent in wakefulness and enables and maintains sleep in rats, dogs and humans[48, 49]. Recently, the results of a phase II study of 161 patients with

primary insomnia were reported[50]. The drug significantly improved the primary parameter of sleep efficiency, objective latency to persistent sleep and wake after sleep onset (WASO), in a dose-dependent manner. As compared with placebo treatment, 400 mg of almorexant given just before the sleep period significantly increased sleep efficiency (+14.4 %), and significantly decreased latency to persistent sleep (–18 min) and WASO (–54 min) on treatment nights. Notably, subjective sleep qualities were also significantly improved by almorexant. Effective doses of almorexant did not cause any relevant negative effects on next-day performance, as is commonly observed with current insomnia treatments such as BzRAs. As expected, almorexant slightly increases time spent in REM sleep, and slightly shortened the latency to enter REM sleep. Importantly, however, almorexant was well tolerated with no signs of cataplexy, hypnagogic hallucinations and sleep paralysis, suggesting that acute and short-lived blockade of orexin receptors does not cause narcolepsy-like symptoms. Since 2008, Actelion and GlaxoSmithKline (GSK) have been conducting phase III studies with almorexant. However, in January 2011, they discontinued these studies due to the occurrence of adverse effects ,[63] which are irrelevant to the function of the orexin system.

GSK initially focused on the development of OX1R-selective antagonists: SB-334867 is the first among such compounds and among entire orexin receptor antagonists[51, 52], followed by SB-408124, SB-410220 and SB-674042[53]. These OX1R-selective antagonists may have the potential for treating obesity, drug addiction and panic disorder[1]. GSK also developed SB-649868[54], a dual OX1R/OX2R antagonist that has been reported to promote REM and non-REM sleep in rats and marmosets and its phase II clinical trial has been completed[64] (Table 1). Importantly, this compound has been demonstrated in healthy humans to exert hypnotic activity without inducing noticeable changes in the EEG power density of non-REM sleep, which is in clear contrast to BzRAs that alter the non-REM sleep EEG,

confirming that sleep induced by OX1R/OX2R antagonists has a feature very similar to that of spontaneous sleep[55].

Merck has developed a series of antagonists for orexin receptors in several distinct structural classes[47]. Among them, suvorexant (MK-4305) is the most advanced in terms of clinical pharmacology. Suvorexant is a compound from the diazepane series with a selective and potent DORA activity (Table 1)[56]. Two pivotal phase III efficacy trials for suvorexant have been completed [65,66]. According to the announcement by Merck in June, 2012[67] , suvorexant significantly reduced the time it took primary insomnia patients to fall asleep and increased the time that patients stayed asleep as early as the first night and after daily dosing for 3 months compared with placebo (1,021 patients in trial 1 and 1,009 patients in trial 2). The endpoints for the studies included both subjective and objective measures of sleep onset and sleep maintenance. At 3 months in trials (383 and 387 patients were treated with the high dose at 30 or 40 mg, and 384 and 383 patients were treated with placebo in trials 1 and 2, respectively), daily suvorexant significantly reduced latency to persistent sleep (−10.3 and −21.7 min in trials 1 and 2, respectively) and WASO (−38.4 and −42.0 min in trials 1 and 2, respectively) as compared with placebo treatment. Neither serious drug-related adverse events nor significant next-day objective residual effects were observed. Furthermore, no rebound insomnia of clinical concern was reported upon stopping suvorexant. Importantly, as in the case of almorexant, no sign of narcolepsy-like symptoms, such as cataplexy, was observed. The New Drug Application (NDA) for suvorexant has been accepted by the US FDA and is awaiting approval[72]. In addition, Merck has further developed MK-6096[57], which is another structurally distinct, piperidine-derived, dual OX1R/OX2R antagonist and its phase II clinical studies for insomnia have been completed[68] (Table 1).

Emergence of suvorexant and other orexin-based hypnotics does not mean the end of BzRAs, which globally reduce brain activity. Some patients, for example those with acute sleep

problems due to, for instance, traumatic incident, may still prefer their use, since BzRAs are more powerful than suvorexant in terms of promoting sleep. But in chronic primary insomnia, suvorexant may be the better choice as it promotes more natural sleep without serious adverse events. In addition, suvorexant is unlikely to have any addictive qualities, because blockade of orexin signalling will be expected to rather reduce mesolimbic dopaminergic tone[1].

Orexin receptor antagonists that have entered into clinical studies thus far are all dual OX1R/OX2R antagonists. On the other hand, there is controversy regarding whether OX2R-selective antagonists may be better hypnotics as compared with dual antagonists. A study reported that an OX2R selective antagonist JNJ-10397049, developed by Johnson & Johnson, has better ability to promote non-REM sleep than the dual antagonist almorexant in rats[58]. In contrast, another recent study led to a different conclusion. It reported that an OX1R-selective antagonist SB-334867 produces small increases in REM and non-REM sleep, and an OX2R-selective antagonist EMPA, developed by Hoffmann-La Roche, produces a significant increase in non-REM sleep[59]. But administration of almorexant increases non-REM sleep more than these subtype-selective antagonists, leading to the conclusion that dual orexin receptor antagonism is more effective for sleep promotion than subtype-selective antagonism[59], although the difference in the capabilities of these drugs to cross the blood-brain barrier might influence their effectiveness. The latter view of the superiority of dual antagonists is also consistent with the conclusion derived from studies using subtype-specific knockout mice that OX2R plays a pivotal role, but OX1R has additional effects on promotion of wakefulness[11, 16]. The gating of REM sleep is likely to crucially involve both receptors, and deletions of both receptors are required for frequent occurrence of cataplexy in mice. From this point of view, OX2R-selective antagonists may have a lower risk to cause cataplexy when taken by insomnia patients. However, this point seems dispensable since, as a matter of fact, clinical trials have shown that daily dosing of dual antagonists at night does not cause cataplexy. Cataplexy may be

a pathological condition that emerges after almost complete loss of orexin signalling for a prolonged period, as in the case of human narcolepsy. In any case, further studies are needed to answer whether OX2R-selective antagonists are better hypnotics or not.

Where are the action sites of the orexin receptor antagonists for their anti-insomnia effects? As described above, pharmacological application of orexin peptides activate wake-active monoaminergic and cholinergic neurons[1]. However, which neurons among them play important roles in the regulation of physiological sleep/wakefulness by endogenous orexins in vivo have remained uncertain. Histaminergic neurons in the TMN, which express OX2R exclusively[11], may be a candidate of such neurons, since histamine-1 receptor is required for arousal effects of ICV orexin-A administration[26, 30] and OX2R expression in the posterior hypothalamus including the TMN rescues sleepiness in narcoleptic OX2R-deficient mice[60]. On the other hand, optogenetic activation of orexin neurons promotes wakefulness in mice lacking histamine[61], denying roles of histaminergic neurons as an efferent pathway of orexin neurons. Instead, activity of noradrenergic neurons in the LC has been reported to be required for the arousal effect of optogenetic stimulation of orexin neurons, suggesting these neurons as another candidate efferent pathway[62]. However, this perspective is challenged by the fact that mice lacking OX1R, the subtype exclusively expressed by LC noradrenergic neurons[11], do not show any overt abnormality of sleep/wakefulness[16]. Of course, since orexin receptors are expressed throughout the brain, orexin antagonists may exert their anti-insomnia effects by acting on brain regions other than the TMN and LC, and also by binding to multiple sites involved in sleep/wake regulation. Future studies by means of focal administrations of orexin receptor antagonists and focal deletion of orexin receptor genes would answer this question.

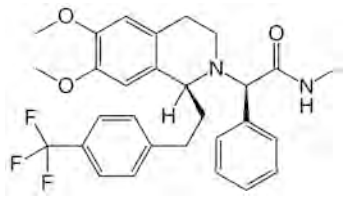
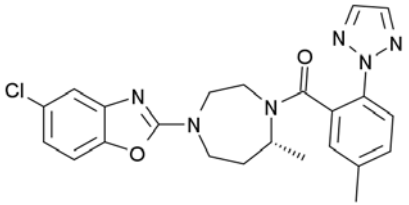
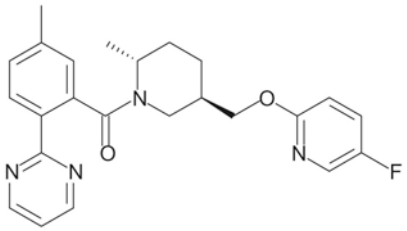
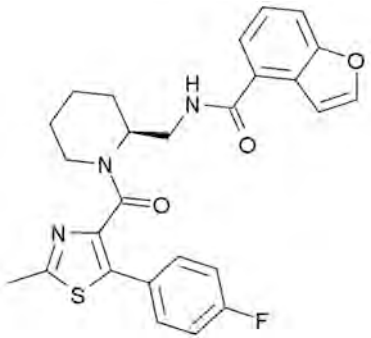
6 Conclusion

The discovery of a critical role played by the orexin system in the regulation of sleep/wakefulness has opened the door of a new era for sleep medicine. Orexin receptor antagonists appear to have a good chance of success as a new medication for primary insomnia, as demonstrated by almorexant and suvorexant. On the contrary, for treating daytime sleepiness such as narcolepsy, development of bioavailable orexin receptor agonists is awaited. Allosteric enhancers for orexin receptors may be satisfactory as a novel medication of daytime sleepiness caused by reasons other than the loss of orexin neurons. In the case of agonists/enhancers, however, potential risk of addiction should be taken into account, since orexin signalling potentiates the mesolimbic dopamine pathway[1]. Lastly, given that the orexin system controls wakefulness through interactions with systems regulating emotion, reward and energy homeostasis, agonists and antagonists for orexin receptors may have a potential for novel treatment of other pathological conditions such as panic disorder, obesity and drug addiction[1]. It should be also noted that phase II trials are investigating the safety and efficacy of MK-6096 for migraine prophylaxis, major depression and painful diabetic neuropathy.[69]

Acknowledgements

The writing of this review was supported in part by Grants-in-Aid for Challenging Exploratory Research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan. The authors declare that there are no conflicts of interest present.

Table 1 Structures of orexin receptor antagonist that have undergone clinical trials for insomnia

Compound	Structure	Clinical trial
ACT-078573 (Almorexant)		Phase III, discontinued
MK-4305 (Suvorexant)		Phase III, completed Filed with the FDA
MK-6096		Phase II, completed
SB-649868		Phase II, completed

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