Chapter 8

Orexinergic System and Antinociception

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Abstract

Orexin-A and orexin-B neuropeptides are specifically synthesized and secreted in hypothalamic neurons. It is known that the orexinergic system participates in pain modulation as well as its roles in various physiological processes such as feeding, stress processing, endocrine and cognitive functions. Orexin ligands activate orexin type-1 (OXR1) and orexin type-2 (OXR2) receptors, each with a different distribution. Orexins are mediators that play important roles in regulating pain perception at the spinal and supraspinal levels. These regulatory roles of orexins have been demonstrated in mechanical, thermal, and chemical models of pain. Periaqueductal gray (PAG) area of the central nervous system plays an important role in the pain modulation of orexins. Furthermore, the orexinergic system locus seroleus (LC) and paragigantocellular lateralis (LPGi) are associated areas and play an important role in chronic neuropathic pain, stress pain and migraine pain as well as opioid analgesic activity. In addition, it has been expressed that the antinociceptive effects of orexins are mediated by endocannabinoids. This review summarizes studies investigating the antinociceptive effects of orexin in various types of pain, including migraine, neuropathic pain, visceral and orofacial pain, and its effects on opioid analgesia and tolerance.

1. Introduction

Orexin-A and orexin-B neuropeptides that make up the orexin (hypocretin) system are produced by enzymatic reactions from prepro-orexin in the lateral hypothalamus. Each of these neuropeptides exert their effects via two G-protein coupled receptors (GPCR) called orexin-1 (OX1) and orexin-2 (OX2). Orexin-A activates both OX1 and OX2 receptors with similar affinity, while orexin-B activates only OX2 receptors (1).

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Orexinergic neurons are located especially in the dorsal medial hypothalamus, perifornical area, and lateral parts of the lateral hypothalamus (2, 3). These neuropeptides have been reported to be involved in a wide variety of physiological functions such as arousal (4), feeding (2), neuroendocrine processes (5), and autonomic control (6). There are two types of G-protein coupled receptors for orexin ligands that are distributed differently in the central nervous system (7). These receptors are orexin type-1 (OX1R) and orexin type-2 receptors. OX1R is selective for orexin-A, while OX2R binds both orexin neuropeptides (2). The widespread distribution of orexin receptors in the central nervous system explains the multifaceted contribution of the endogenous orexinergic system to homeostatic regulation in the brain (2). Abundant evidence suggests that both OX1 and OX2 receptors are expressed on the soma at pre- and post-synaptic terminals, as well as in the medial and lateral hypothalamic areas (8).

Numerous studies have shown that orexins have antinociceptive effects in the brain and spinal cord in different types of pain, including mechanical (tail-pressure), thermal (hot-plate, tail-flick, claw-retraction), chemical (formalin, capsaicin, and abdominal) (9). Orexin receptors can be detected in many brain structures known to be involved in the transmission of pain signals (10). It has been reported that Orexin-A exerts antinociceptive effects in both the brain and spinal cord. In contrast, it is stated that orexin-B has little antinociceptive effect (11). The periaqueductal gray (PAG) area in the central nervous system is an important site in pain regulation by orexins. The analgesic supraspinal mechanism involves retrograde inhibition of GABA release in the ventrolateral PAG. In addition, orexins interact with endocannobinoids to produce analgesia (12).

The purpose of this section is to elucidate the role of orexin, a hypothalamic neuropeptide, modulation of pain transmission, opioid analgesia and tolerance.

2. Mechanisms of Analgesic Effect of Orexin

A large body of evidence provides important insights into the possible mechanisms of orexin's analgesic action. Orexin increases antinociceptive activity in the ventral tegmental area (VTA) by activation of orexinergic receptors in the nucleus accumbens (NAc) in rats (13, 14). Functions related to nutrition, neuroendocrine functions, sensory information and nociception are probably formed as a result of mutual interactions of orexins and neurokinin-1 receptor. This is evidenced by the direct anatomical contact between orexin-A and neurokinin-1 receptors in the marginal layer of the

dorsal horn (15). Another analgesic mechanism of action with Orexin-A is retrograde inhibition by GABA secretion by increasing the production of 2-arachidonoylglycerol (2-AG), an endocannabinoid, in the ventrolateral PAG (16). In addition, a study with orexin-B suggested that activation of glycinergic and purinergic neurotransmission at the spinal level enhanced the analgesic effect of orexin (17).

3. Orexinergic System and Neuropathic Pain

Orexinergic neurons play an important role in chronic neuropathic pain as well as stress pain, headache, migraine pain (Table 1). It is a treatment-resistant chronic pain condition that results from any lesion or disease of the somatosensory nervous system and has adverse effects on quality of life. The role of the orexinergic system in neuropathic pain has been demonstrated in various animal models. The effect of orexin-A on neuropathic pain was evaluated in rats with neuropathic pain created by sciatic nerve ligation. Orexin-A administered intrathecally 7 days after sciatic nerve injury reduced the level of mechanical allodynia and neuropathic pain induced by partial sciatic nerve ligation (18).

It reveals that orexins can reduce heat-induced hyperalgesia in rats with chronic constriction damage to the sciatic nerve. Orexin-A administration inhibited hyperalgesia caused by sciatic nerve damage, while orexin-A antiserum antagonizes this effect (19). In addition, orexin-A inhibits Ca2+ flow through the L-type Ca2+ channel in dorsal root ganglion (DRG) neurons of rat segmental spinal nerve ligation with its effect on high K+-induced [Ca2+]i increase. This indicates an important effect of orexin for nociceptive modulation. In addition, nifedipine and lidocaine potently block high K+-induced depolarization [Ca2+] increase in rats with spinal nerve ligation (20). Furthermore, neuropharmacological evidence indicates that OXR2 mediates its inhibitory effects on KCl-induced increases in [Ca2+]i in C-fiber neurons in a model of sciatic nerve ligation-induced hyperalgesia in rats (21). In a neuropathic pain model in rats, orexin-A exerts its antinociception effect through injection of the cholinergic agonist carbachol and stimulation of the posterior hypothalamus, partially mediated by the OXR1 in the dorsal horn of the spinal cord (22). Intrathecal administration of orexin-A to animals significantly reduced the occurrence of mechanical allodynia experimentally induced by sciatic nerve ligations (18). In addition, intrathecal administration of orexin-A blocked hyperalgesia, a form of neuropathic pain, in streptozotocin-induced diabetes mellitus mice (23). Moreover, orexin neurons show that they modulate the sensations of pain and itching in the opposite direction, namely pain relief and itching

exacerbation (24). In addtion, orexin-A/OX1R signaling has been shown to play an important role in the prevention of central poststroke pain in mice through activation of the descending pain control system (25).

Table 1. Summary of studies on pain modulation of the orexinergic system

Orexin A/Orexin B	**OXR1/ OXR2**	**Pain models**	**Antinociceptive activity**	**Ref.**
Orexin A/Orexin B (i.c.v. and i.t.)	OXR1/ OXR2	Tail-flick, hot-plate test, in mice	Enhanced antinociception	(9)
Orexin A (i.c.v.)	OXR1 agonist	Tail-flick test, in rats	Inhibitory effects of pain on RVM	(26)
Orexin A (intra-PAG injection)	OXR1 agonist	Formalin test, in rats	Decreases nociception	(27)
Orexin A (intra-LPGi)	OXR1	Formalin test, in rats	Decreases nociception	(28)
Orexin A (i.c.v. and i.t.)	OXR1	Sciatic nerve ligation (neuropathic pain model) in rats	Alleviates mechanical allodynia	(18)
Orexin A (i.v.) Orexin B (i.v.)	OXR1 OXR2	Model of trigeminovascular nociception in rats	Orexin A has antinociception Orexin B has no antinociception	(29)
Orexin A	OXR1	Model of trigeminovascular nociception in rats	Inhibit A-fibre responses to electrical stimulation	(30)
Orexin A antagonist	OXR1 (SB-334867)	Formalin test, in rats	Attenuate swim- and restraint stress-induced antinociception	(10)

icv, intrcerebroventricularis; it, intratechalis; LPGi, paragigantocellularis lateralis; RVM, rostral ventral medulla

4. Orexinergic System and Stress Induced Analgesia

The stress-induced analgesic effect is an important form of the defensive behavioral reaction to fight-or-flight (31). Acute and chronic stress affect the orexinergic system, causing changes in both pain threshold and nociceptive behaviors (32). Evidence suggests that chronic stress activates orexin neurons and thereby inhibits pain transmission In addition, stressful conditions in experimental animals appear to increase orexin levels, resulting in improved animal performance and blocking of pain signals (33). Recent evidence has shown that the interaction between corticotropin-releasing factor (CRF) and orexinergic neurons may be physiopathologically related to the control

of stress-related behaviors. Activation of the orexinergic system modulates the activity of CRF neurons via OX2R (7). Many evidences suggest that OX1R responds to both pain and stressful stimuli and is therefore likely to be involved in stress-induced analgesia (34).

During stress, hypothalamic orexin neurons are activated and orexins are released. Increasing 2-arachidonoylglycerol (2-AG) activates the orexin receptor type-1 in the lateral PAG, resulting in analgesia. Stress analgesia has been demonstrated in a mouse model where the orexin and nociceptin/orphanin FQ systems coordinately regulate nociception (31). In an experimental study, the paw thermal nociceptive test showed that restraint of immobilization of the rat increased the pain threshold by 20.5%. Injection of nociceptin/orphanin into the perifornical area of the lateral hypothalamus in rats significantly reduced stress-induced analgesia. It has been reported that the formation of stress-induced analgesia is mediated by direct inhibition of the orexinergic system in the perifornical area (35). Intracerebroventricular injection of the selective orexin receptor type-1 antagonist SB 334867 reduced the analgesic effect of restraint stress in the formalin test. Similarly, blocking of orexin-1 receptors with SB-334867 in rats resulted in a reduction in antinociceptive behaviors induced by swimming and restraint stress in the formalin test (10). Therefore, it can be stated that the orexin receptor type-1 mediates an opioid-independent stress-induced analgesia. In one study, prior administration of the OXR1 antagonist SB 334867 resulted in a reduction in the antinociceptive effects of restraint stress in animals (36).

In addition, another study demonstrated the role of orexin receptors in antinociception induced by swimming or restraint stress. This study showed that exposure to 6 minutes of swimming stress combined with 30 minutes of restraint stress can significantly reduce formalin-induced nociception in rats. The analgesic effect caused by restraint stress or swimming stress was fully inhibited by the orexin-1 receptor antagonist SB-334867 (10).

5. Orexinergic System and Headache

Migraine is defined as a diffuse, chronic, debilitating neurovascular disorder associated with sensory sensitivity, usually manifested by severe unilateral headaches. Cluster headache, on the other hand, is a disease that starts with recurrent unilateral pain attacks, is severe, and is often associated with autonomic symptoms (37). In general, cluster headache and migraine are expressed as the two main primary headache disorders. The basic mechanism in the occurrence of migraine includes the activation of the trigeminovascular system. Orexin-A prevented electrical stimulation-

induced vasodilation, which was prevented by SB-334867 pretreatment. In addition, orexin-A blocks the prejunctional release of calcitonin gene-related peptide (CGRP), which is very important in migraine, from trigeminal neurons (38).

Furthermore, it has been determined that the activation of the hypothalamus has an important role in the physiopathology of cluster headache. Cluster headache manifests itself with circadian or seasonal attacks and shows some features such as changes in hormone levels (37). Orexin neurons are highly organized in the hypothalamus and show broad projections to areas involved in nociception and autonomic regulation. Given these features of the orexinergic system, orexins are likely to play an important role in the pathogenesis of cluster headache and migraine. Injection of orexin-A into the posterior hypothalamic region of the rat reduces A- and C-fiber type nerve responses to dural electrical stimulation in the trigeminal nucleus caudalis and harmful thermal stimulation of the facial receptive field (39). Intravenous administration of orexin-A has been shown to inhibit neurogenic dural vasodilation through OXR1 activation. This effect acts on the trigeminovascular system, causing partial blocking of calcitonin gene-related peptide (CGRP) release (29). In another study, intravenous injection of orexin-A was shown to block type-A nerve fiber responses to dural electrical stimulation through activation of OXR1 (30).

Although many studies support that the orexinergic system has a very important role in the pathophysiology of migraine, the results of a clinical study show that the orexin receptor antagonist fluorexant does not have sufficient analgesic effect as a potential therapeutic approach in migraine (40).

6. Orexinergic System and Visceral Pain

Visceral sensation is one of the main functions of the gastrointestinal tract and is controlled by the central nervous system. The modulatory role of orexins secreted from the brain in visceral sense indicates that the orexinergic system may play a role in the pathophysiology of irritable bowel syndrome (41). According to a study, intracisternal injection of orexin-A caused an increase in the threshold volume of the abdominal withdrawal reflex due to colonic distension. At the same time, the threshold volume was not changed by the intracisternal OXR1 antagonist SB334867, while centrally administered SB334867 completely blocked the morphine-induced analgesic effect against colonic enlargement (42). The results obtained in this study show that orexin-A can play a modulatory role by being secreted from the central nervous system to prevent pain caused by colonic distension.

Furthermore, dopaminergic signaling pathways have also been found to play an important role in orexin-induced central analgesic activity against colonic distension (43). In addition, prior administration of the D1 dopamine receptor antagonist SCH23390 to animals prevented the analgesic effect from centrally injected orexin-A for colonic distension (44). Moreover, it has been demonstrated that the adenosine signaling system also plays an important role in visceral antinociception regulated by the orexinergic system. Evidence suggests that subcutaneous administration of theophylline, an adenosine antagonist, or 1,3-dipropyl-8-cyclopentylxanthine, an adenosine A1 receptor antagonist, against colonic distension caused by the centrally injected A1 agonist N(6)-cyclopentyladenosine (CPA) or orexin-A. demonstrated that it inhibits antinociceptive activity (44).

5. The Role of the Orexinergic System on Morphine Analgesia and Tolerance

Opioid drugs such as morphine are frequently used in the clinic for the treatment of chronic and severe pain. On the other hand, long-term use of these drugs often causes tolerance to analgesic effects (45). There are several possible explanations for the development of morphine tolerance, including activation of an intracellular signaling pathway such as nitric oxide (NO) and mammalian rapamycin target (mTOR), apoptosis in dorsal ganglion neurons, ghrelin and opioid receptor desensitization, and endocannabinoid receptor induction (46-49). Numerous studies have shown that OXR1 antagonist administration causes a decrease in the development of morphine-induced tolerance (50-52) (Table 2).

Co-administration of OXR1 antagonist SB-334867 with morphine inhibits the development of opioid tolerance. Therefore, these data suggest that OXR1 plays an important role in the development of tolerance to morphine (51).

Table 2. The role of the orexinergic system on morphine analgesia and tolerance

Orexin A/ Orexin B	**OXR1/ OXR2**	**Pain models**	**Morphine analgesia and tolerance**	**Ref.**
Orexin A antagonist	OXR1 antagonist (SB-334867)	Tail-flick, hot-plate test, in rats	Attenuates morphine tolerance	(52)
Orexin A antagonist	OXR1 blockade (SB-334867)	The electrical activity of LC neurons was studied using single unit recording in rats	Prevents morphine tolerance	(53)
Inhibits orexin A secretions (Yokukansan)	No receptor blockade	Hot-plate test, in rats	Attenuates morphine tolerance	(54)
No effect	OXR1/OXR2 antagonist suvorexant	Tail-flick test in mice	Reduces morphine tolerance and dependence	(55)
No effect	OXR1 antagonist (SB-334867)	The electrical activity of LC neurons was studied using single unit recording in rats	Attenuates morphine tolerance	(56)
Orexin B	OXR2 agonist	Tail-flick, hot-plate test, in rats	Attenuates morphine tolerance	(52)
No effect	OXR1 antagonist, icv (SB-334867)	The electrical activity of LPGi neurons was studied using single unit recording in rats	Prevents morphine tolerance	(57)
No effect	OXR1 antagonist, icv (SB-334867	Transparent cylindrical plexiglas test chamber, in rats	Decreases naloxone precipitated morphine withdrawal signs.	(58)
No effect	OXR1 antagonist, icv (SB-334867	Transparent cylindrical plexiglas test chamber, in rats	Decreases naloxone precipitated morphine withdrawal signs.	(59)
No effect	OXR1 antagonist, icv (SB-334867	The warm-water tail immersion test, in rats	Attenuates morphine tolerance and dependence	(60)
No effect	OXR1 antagonist, icv (SB-334867	Tail-flick test, in rats	Attenuates morphine analgesic tolerance	(51)

icv, intrcerebroventricularis; LC, locus coeruleus; LPGi, paragigantocellularis lateralis

In addition, orexins play a modulatory role in the analgesic effect of met-enkephalin in locus coeruleus (LC) neurons. The nucleus locus coeruleus is one of the important central nervous system regions in the analgesic activity of opioids and the development of tolerance to morphine. However, they are detected in high concentrations in LC neurons of the orexin receptor type-1 (61). Spinal administered orexin-A has been shown to reduce mechanical hyperalgesia caused by repeated intradermal injections of the mu opioid receptor agonist DAMGO via OX1R (62). Inhibition of OXR1 with SB-334867 induced a significant alleviation of the development of morphine dependence and behavioral symptoms induced by naloxone administration in rats. In addition, long-term blockade of OXR1 may reduce formalin-induced nociception (63).

In one study, intracerebroventricular administration of the OXR1 antagonist SB-334867 to rats demonstrated tolerance to the nociceptive effect of morphine and a reduction in naloxone-induced withdrawal symptoms. Therefore, orexins may play a role in morphine analgesic tolerance and dependence via OXR1 receptors (60). In another study, when the selective orexin receptor-1 antagonist SB-334867 was administered to rats in the locus cereleus, morphine reduced analgesic tolerance and the development of physical dependence (59). In an electrophysiological study, it was shown that blockade of orexin type-1 receptors prevented the development of morphine tolerance in the lateral paragigantocellularis nucleus of rats (57). Similarly, blocking the orexin type-1 receptor in the lateral paragigantocellularis nucleus in rats has been shown to reduce naloxone-induced morphine withdrawal symptoms (58). In two studies at different times, the central inhibition of orexin type-1 receptors with an OXR1 antagonist showed significant reductions in activation of locus coeruleus (LC) neurons in morphine-dependent rats with naloxone (56, 64). In an electrophysiological study, it was demonstrated that OX1R blockade by SB-334867 in rats prevented the development of morphine tolerance in LC neurons (53). Administration of suvorexant, an OXR1 antagonist, to mice reduces morphine-induced tolerance and dependence. In addition, with repeated administrations, morphine increases tolerance and dependence in mouse brain through elevation of NMDA, p-ERK and CREB protein levels. Suvorexant prevents opioid tolerance and addiction by blocking orexin receptors in the brain and lowering p-ERK and CREB protein levels (55).

Bilateral administration of the OX1 receptor antagonist into the nucleus accumbens (NAc) showed that morphine sensitization acquisition was reduced, but the OXR2 antagonist produced similar effects only at the

maximal dose. These findings suggest that the OX1 and OX2 receptors are in the NAc and play a role in acquiring morphine sensitivity (65). Yokukansan, a traditional herbal (Kampo) drug consisting of seven components, has been shown to reduce morphine tolerance through inhibition of orexin-A secretion (54). In an experimental study, it was reported that orexin-A may participate in the expression of naloxone-induced morphine withdrawal syndrome by partially reducing the activity of neurons carrying $GABA_A$ receptors (66). In addition, co-administration of OX1R antagonist SB-334867 and OX2R agonist orexin-B with morphine to rats decreased morphine tolerance in tail-flick and hot-plate antinociceptive tests (52).

6. Conclusion

Numerous evidences confirm that orexinergic neurons show antinociceptive activity at both spinal and supraspinal levels in various types of pain, such as neuropathic pain, migraine pain, stress pain, and headache. According to the literature, orexin-A shows its antinociceptive activity by OX1 receptors and this activity is more than orexin-B. Although many mechanisms have been suggested, the antinociceptive action mechanisms of orexins have not been fully elucidated. Ventrolateral PAG is an important site of pain modulation of orexinergic neurons and plays a role in supraspinal regulation. Retrograde GABA secretion has an important role in this supraspinal mechanism. In addition, clinical studies investigating the role of the orexinergic system in pain processing are mostly related to the treatment of migraine, cluster headache, chronic neuropathic pain, and stress pain. The results of the studies showed that orexin receptors, especially OXR1, play an important role in morphine analgesic tolerance and dependence.

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