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Review Article

Neurokinin 1 and opioid receptors: relationships and interactions in nervous system

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Abstract:

Opioid receptors and neurokinin 1 receptor (NK1R) are found highly expressed in the central nervous system. The co-localization of these two kinds of receptors suggests that they might interact with each other in both the transmission and modulation of the pain signal. In this review, we explore the relationships between opioid receptors and NK1R. Substance P (SP) plays a modulatory role in the pain transmission by activating the NK1R. Opioid receptor activation can inhibit SP release. NK1R is found participating in the mechanisms of the side effects of the opioids, including opioid analgesic tolerance, hyperalgesia, anxiety behaviors of morphine reward and opioids related respiratory depression. A series of compounds such as NK1R antagonists and ligands works on both mu/delta opioid receptor (MOR/DOR) and NK1R were synthesized as novel analgesics that enhance the clinical pain management efficacy and reduce the dosage and side effects. The current status of these novel ligands and the limitations are discussed in this review. Although the working mechanisms of these ligands remained unclear, they could be used as research tool for developing novel analgesic drugs in the future.

Key words: Opioid receptors, Neurokinin 1 receptor, Substance P, Opioids, Central nervous system

Pain is common in many medical conditions, and can reduce the quality of life.[1] Pain is also the major reason for physician consultation in most countries.[2] Pain management is always a challenging for physicians to help the patients suffering from the pain. Analgesics are the most common and effective management in many different kinds of pain. Opioids are the most commonly used and effective drugs for severe pain in the clinical practice despite that they have severe side effects including death and addiction. In 2013, 0.6% to 0.8% of the global population (between 28 and 38 million) between the ages of 15 and 65 used opioids recreationally, based on the world drug report of UNODC (United Nations Office on Drug and Crime, https://www.unodc.org/).

Opioids exert their pharmacological effect by binding to opioid receptors, which are distributed widely in the brain, spinal cord and the peripheral sensory neurons and tissues. There are three classic opioid receptors including mu (MOR), delta (DOR), and kappa (KOR) opioid receptors. Each kind of these receptors has the unique pharmacological features. They could interact with other receptors including the neurokinin 1 receptor (NK1R), another important receptor related to pain signal transmission. Recent studies indicate that NK1Rs co-localize with the opioid receptors in the nervous system. To activate or block the NK1R may interfere with the function of the opioids and their receptors.

Both opioid receptors and NK1R belong to G protein coupled receptors (GPCRs) family.

Substance P is the endogenous ligand for NK1R also known as tachykinin receptor 1 (TACR1) or substance P receptor (SPR). SP is a neuropeptide present in unmyelinated primary afferents and is released in response to pain or noxious stimuli.[3] When SP is released and bound to the NK1R, it triggers the transmission of stress signals and pain, which is associated with the contraction of smooth muscles and inflammation. NK1R antagonists have been studied in migraine, emesis and psychiatric disorders. They are also explored to be used to manage the side effect of opioids.

Both opioid receptors and NK1R are highly expressed in the central nervous system.[4] The co-localization of these two kinds of receptors suggests that they play important roles in the direct and indirect control of pain signal transmission and modulation.[5] In this review, we will explore the relationship between NK1R and opioid receptors and review potential ligands for pain management targeting both NK1 and opioid receptors.

SP and opioids on pain modulation

SP and opioids have opposite effects on pain behavior, the nociceptive effect of the former being balanced by the antinociceptive effect of the latter. SP is a neuropeptide, acting as a neurotransmitter and a neuromodulator, may play a modulatory role in pain transmission.[6] Studies indicated that the response to intense pain was significantly reduced in preprotachykinin A gene knockout mice, while the behavioral response to mildly painful stimuli was unchanged.[7,8] Neurogenic inflammation, which results from peripheral release of SP was almost absent in the mutant mice.

Fukazawa et al. revealed the involvement of SP in the mechanism of cholecystokinin (CCK)-induced anti-opioid effects.[9] Electroacupuncture (EA)-induced activation of spinal CCK might increase the release of SP from the primary afferent terminal. Activation of the NK1R was markedly involved in the CCK-induced attenuation of morphine analgesia. Pretreatment with the NK1R antagonist could reverse EA- and CCK-induced attenuation of morphine analgesia. The release of SP and subsequent activation of the secondary afferent neuron by SP might be the key mechanism of the attenuation of morphine analgesia following EA stimulation and intrathecal administration of CCK.

A recent study has shown that SP may reprogram the MOR recycling and signaling through activation of NK1R in trigeminal ganglion (TG) neurons.[10] SP increased the recycling and enhanced the resensitization of MOR after fentanyl administration. Their result defined a physiological pathway that cross-regulates opioid receptor recycling via the direct modification of MOR. While most of the studies reported SP as a nociceptive factor that could cause pain and be involved in pain transmissions, opposite findings were also reported. When SP was microinjected into the ventrolateral periaqueductal gray (PAG) in rats, it significantly increased the hindpaw withdrawal latencies (HWLs) to thermal and mechanical stimulation. Such effects could be blocked by NK1R antagonist.[11] SP injection into the ventrolateral PAG induced an antinociceptive effect via the activation of NK1R in the central nervous system. Systemic morphine administration induced the release of SP in the ventrolateral PAG. These results suggest that SP may have distinct functions in different parts of the brain, spine and peripheral neuron system. Thus, SP may play an important modulatory role on pain transmission.

Opioid Receptors modulate SP release

MOR activation may inhibit SP release. One of the mechanisms of opioid analgesia is inhibition of the release of SP from the presynaptic afferent terminals in the spinal dorsal horn.[12] Thomson et al. reported that the immunoreactivity for SP as well as the expression and function of NK1R in the dorsal horn of the morphine treated neonatal rats were decreased.[13] MOR and its ligands may either inhibit the release of SP or reduce the responses of the second order neurons to SP. Morphine's efficacy can be regained by NK1R antagonists, indicating that the anti-morphine reaction of SP is via activation of NK1R.[10] While it was also reported that morphine reduced only minimal SP-induced NK1R internalization. Morphine withdrawal can induce NK1R internalization by the induction of SP release and activation of the NK1R.[14] Although, the mechanism is still not yet clear, NMDA receptors could be involved in this process.[15]

DOR activation may also affect the SP release. Activation of DOR in the superficial dorsal horn blocks the formalin-induced SP release and NK1R internalization. [16] Intrathecal administration of SNC80, a DOR agonist, inhibited formalin-induced NK1R internalization, and such inhibition could be reversed by naltrindole, a DOR antagonist. This study showed the effect of intrathecal SNC80 on SP release and the results were consistent with the observations reported by Joseph and Levine [17], while the were different from those reported by Scherrer [18] and Riedl et. al.[19] However, these studies collectively suggested that DOR agonists could suppress the excitability of primary afferent terminal and block the SP release.[20-22]

KORs modulate the release of SP in the dorsal horn. A study found that dynorphin(1-8), highly selective KOR ligand, reduced the basal release of SP when applied to the spinal cord of rats.[23] KOR agonist prevented the increase in the release of SP in the rat spinal dorsal horn, which was activated by peripheral nociceptors of thermal stimulus.

An immunobiological studied have demonstrated that MOR and DOR agonists could prevent afferent-evoked NK1R internalization indicating that the release of SP from the primary afferents was inhibited, which subsequently contributed to intrathecal opioids analgesia. [24] Further study found that no matter formalin- or capsaicin-induced SP release in rat spinal cord could be blocked by MOR and DOR agonist based on functional assay (animal behavior and NK1R internalization) in vivo.[8]

Study involving different pain models reported that the inhibitory effect of opioids on SP release from primary afferents disappeared in the spinal dorsal horn in neuropathic pain model, but maintained in inflammatory pain model.[25] In the neuropathic pain model, there is a complete loss of the ability of MORs to inhibit SP release in the dorsal horn. In contrast, inflammation of the hindpaw with CFA did not affect the ability of the MOR agonist DAMGO to inhibit SP release. This indicated that MOR signaling pathway might be inhibited resulting in the lack of ability to suppress SP release in neuropathic pain.

Morphine could enhance NK1R expression in the cortical neurons at both mRNA and protein levels, and morphine treated cortical neurons could increase the SP induced calcium mobilization. Blockade of opioid receptors on the cortical neurons by naltrexone abolished the morphine induced SP changes. [26]

The blockade of the opening of voltage gated calcium channels might be responsible for the transmitter (G-protein beta gamma subunits) release. This inhibitory action was mediated by activation of the Gi/Go protein and the interaction of its β/γ subunits of the calcium channels.[27]

SP and NK1R effect on the opioid analgesic tolerance

The understandings about the molecular processes responsible for loss of MOR function after chronic exposure to opioids is still unclear. The elucidation of cellular mechanisms are very important for the successful development of opioids based pain therapeutics without tolerance.[40] The morphine tolerance is a condition that has been characterized by a decreased sensitivity to morphine treatment.[28] Experiments have proven that SP-NK1R activation may induce central sensitization to pain.[29-31] Data suggested that SP acting at NK1R may heightened pain states resulting from both inflammatory [32,33] and neuropathic pain [34,35]. The role of SP-NK1R activation was studied and found that NK1R internalization and expression was increased in morphine tolerant rats.[36] Thomson et al also reported a loss of SP-NK1R function in the spinal cord dorsal horn in rats exposed to repeated doses of morphine.[13] They demonstrated that the expression of NK1R was down-regulated in the spinal cord in morphine-treated rats. These results suggest that repeated morphine exposure, which develops into a morphine analgesic tolerance, profoundly alters SP and NK1R expression and function in the spinal cord dorsal horn.

Recreational morphine exposure might have different effects on NK1R expression.[37] The up-regulation [36] and the down-regulation of the NK1R [13] reflected different stages of a same process with increase signaling of SP. Increased neuropeptide SP signaling may indicate an important mechanism of the morphine analgesic tolerance. In cultured adult dorsal root ganglion neurons, the exposure to the NK1R antagonist could both block and reverse the development of morphine tolerance.[38] Thus, neuropeptide activity contributing

to tolerance was existed at the level of the primary afferents terminating in the spinal cord. Opioid analgesic tolerance was also depends on compensatory or opponent processes to desensitization or internalization of the opioid receptors. The mechanisms of opioid analgesic tolerance are complicated and may also represent important therapeutic targets for the pain relief with prolonged opioid analgesic treatment.

Base on the theory that neuropeptide SP is an important regulatory effector of opioid-dependent analgesic processes, ESP6, a SP-opioid chimera, was synthesized.[39] ESP6 contained an overlapping domains of endomorphin-2 and SP. Intrathecal administration of ESP6 with morphine leaded to a prolongation of morphine analgesia over a 5-day period. The analgesia induced by ESP6 and morphine was opioid receptor dependent since naltrexone could block the analgesic effects. Furthermore, when ESP6 and morphine were co-administered with NK-1 receptor blockade, a decrease of analgesic potency was observed similar to that of morphine administration alone. This represented a novel strategy for prolonging opioid analgesic effects.

NK1R neurotransmission induces hyperalgesia

NK1R mediates both the chronic thermal hyperalgesia and the decreased efficacy of opioids. The pain behavior using mice lacking noradrenaline (NA) was studied. It was observed that absence of NA in central nervous system resulted in a decreased nociceptive threshold to thermal but not mechanical stimuli, and the reduced opioid efficacy associated with a lack of NA was due to increased NK1R stimulation.[40] Related mechanism was associated with the development and maintenance of hyperalgesia during sustained opioids exposure including the activity of NK1R expressing ascending spinal neurons and descending pathways originating in the rostral ventromedial medulla (RVM). These mechanisms may also be important in opioid enhancement of pain management during the surgery. It was found that the descending facilitator pathways played an important role, while NK1R containing ascending pathways only played a partial role in opioids induced hyperalgesia and in the enhanced hyperalgesia induced by fentanyl following surgical incision.[41]

NK1R modulate morphine reward and anxiety behaviors

NK1R is highly expressed in central nervous system. It is especially involved in depression, anxiety and stress. The amygdale is an important area for the effects of SP and NK1R in the motivational properties of opioids and the control of behaviors related to anxiety. NK1R activation can influence opioid reward specifically. NK1R knockout mice lacks many behaviors associated with morphine reward.[42] NK1R activation by SP inhibited the MOR endocytosis, such inhibition is observed in both amygdale and locus ceruleus neurons which co-express NK1R and MOR naturally, and is a nonreciprocal action.[43] The regulation of MOR trafficking by NK1R is associated with reduced desensitization of adenylyl cyclase signaling in striatal neurons. These cell autonomous suggest the specific effects of NK1R on opioid signaling.

NK1R mediate sex difference in opioids-enhanced contract hypersensitivity (CHS)

CHS is a type of cutaneous inflammation that is exacerbated by neurogenic factors. Morphine administration prior to the challenge with antigen 2,4-dinitro-fluorobenzene increases the CHS response in rats. Study indicated that central opioid receptors and peripheral SP were involved in the morphine induced enhancement of the CHS response.[44] Both MOR and DOR agonists might heighten CHS especially in females. A clinical study reported that potentiated NK1R signaling following opioid treatment accounted for sex differences in the clinical manifestation of CHS.[45] A NK1R antagonist SR140,333 was administrated after morphine treatment and the clinical manifestation of CHS was significantly attenuated in females but not in males. These data suggested that NK1R signaling is a key mediator of sex differences in opioid-induced enhancement of CHS.[45]

NK1R and opioid inhibitory effect on the breathing activity

Opioids have many severe side effects including respiratory depression. The mechanisms are still unclear. A recent study reported that two types of NK1R immunoreactive neurons were found in the pre-BötC,[46-49] which was proved to be a crucial center for the generation of normal breathing in adult mammals.[50] Type 1 neurons expressed both NK1R and MOR. This type of contact provided a possible morphological base

for NK1R immunoreative neurons participated in the modulation of the respiratory function. Glutamate, SP and GABA neurons were widespread in the rat nucleus tractus solitaries.[51] Endomorphin-2 (a endogenous opioid) immunoreative fibers were found to be co-localized with the SP but rarely with glutamate or GABA in the pre-BötC. All these data suggested that NK1R might participate in the modulation of the MOR induced respiratory depression.

NK1R antagonists: potential analgesics

The nociceptive effect of SP can be offset by opioids and restored by NK1R antagonists. Scientists proposed that NK1R antagonists might be considered as an adjunct therapy in chronic pain management. The NK1R blockade might be able to reduce opioid reinforcement, tolerance, physical dependence and withdrawal.[52,53] In preclinical animal studies, NK1R antagonists could effectively attenuate the nociceptive responses caused by inflammation or nerve damage.[54] But unfortunately, NK1R antagonists had failed to exhibit efficacy in clinical trials of a variety of clinical pain states. Thus, some researchers believed that NK1R antagonists appeared to block behavioral responses to nociceptive stimuli only at a level detectable in animal experiments, but failed to provide the sensory blockade to produce clinical analgesia in humans. [55]

Aprepitant, a selective NK1R antagonist that was used as an anti-emetic,[56] was found to possess opioid like effects in patients who maintained and withdrawn from methadone.[57] NK1R antagonist showed some ability to regulate the reactions of methadone withdrawal. Other studies reported that higher doses of aprepitant might be more clinically effective.[58,59] However, further studies are needed to elucidate the mechanisms.

Co-localization of opioid receptors and NK1R

Several studies found that NK1Rs co-localized with opioid receptors in the central nervous system.[4,5] Such coexistence of the receptors indicated direct or indirect interactions between these receptors, and NK1R ligands might act on the same postsynaptic sites in nociceptive neurons. Studies had shown that, in fact, opioid receptor ligands can alter the internalization of NK1R evoked by either noxious stimuli or exogenously given SP, and opioid receptor ligands altered the postsynaptic effects of SP agonism on second-order neurons, but did not alter the binding of SP to the NK1R.[14]

A MOR-NK1R complex was designed to investigate their interaction in nociceptive brain regions.[60] The MOR and NK1R were co-expressed on the same cell. Both ligands induced the recruitment of β-arrestin to the plasma membrane and co-internalization of NK1R-MOR heterodimers into the endosomal compartment. It was discovered that NK1R-MOR heterodimerization altered internalization and resensitization profile of these receptors. The ligand binding and signaling properties were not changed. The physical interaction of the MOR and NK1R was sequestered via the endocytotic pathway with delayed recycling and resensitization kinetics.

New ligands work on both MOR/DOR and NK1R

Based on the current understandings of opioid receptors and NK1R, the researchers aim to work on combining a NK1R antagonists with an opioid receptor agonists, which could target two receptors at the same time.[61,62] Such compound is supposed to provide a new method enhancing the clinical pain management efficacy of opioids by reducing the dosage and risk of opioids side effects. This is achievable because NK1R and opioid receptors are co-localizing in the central nerve system.

Recently, TY005, a dual peptidic opioid agonist-NK1R antagonist was synthesized and evaluated for the efficacy of this compound in thermal and tactile stimuli in nerve injured male rats.[61] The opioid agonist activity and NK1R antagonism were studied in independent assays. Receptor activities were isolated when the other receptor was blocked. It was discovered that this compound was able to carry out the desired dual activity *in vivo*. It was also demonstrated that this multimodal ligand worked well in suppressing antihyperalgesic tolerance. Later, this compound was further optimized by improving opioid agonism and maintaining NK1R activity.[63] Unfortunately, such dual opioid agonist-NK1R antagonist still share some opioid side effects especially the opioid induced tolerance. [64]

In the last decade, a dual peptide was designed for treatment of pain.[63,65-70] Such bifunctional peptide contained a modified C-terminus in which a mu/delta

opioid agonist and a NK1R antagonist were fused into one molecule. In order to optimize for better activities at both mu/delta opioid receptors (MOR/DOR) and NK1R, a series of compounds were tested and a structure-activity relationships study was performed. Fused positions of compounds were found to act as an "address region" for both opioid agonist and NK1R antagonist. With optimization, compounds showed potent activities as an opioid agonist and NK1R antagonist and had promising analgesia for treatment of various pain conditions.[66] *In vivo* studies of the dual peptides showed that two pharmacophores did not work independently and their conformation "balance" greatly impacted on their biological behaviors.[63] Further investigations moved on and new compounds were developed.[67] It was observed that different appropriate truncation of peptide sequence could lead to more effective binding as well as functional activities for both MOR/DOR and NK1R.[68]

Novel opioid agonist and NK1R antagonist bivalent ligands were also synthesized and evaluated.[71] For example, a new carboxy-derivatives of fentanyl (1a–1c) were developed. This compound exhibited MOR agonist and NK1R antagonist activities and might serve as a useful lead compound for the further design of a new series of candidates with dual opioid agonist-NK1R antagonist effects.

Conclusions

Both SP-NK1Rs and opioid receptors are important for the pain modulation and co-localize in the nervous system. SP plays a modulating role in the pain transmission by activating the NK1Rs. Opioid receptor activation could inhibit SP release from the primary afferents. NK1R antagonists could modulate some of the side effect of the opioids, including opioid analgesic tolerance, hyperalgesia after opioids, morphine reward, anxiety behaviors and respiratory depresses. NK1R antagonist and ligands effective for both MOR/DOR and NK1R are under development for either potential novel analgesic medications for pain management or research purposes.

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