The mechanism of \(\mu\)-opioid receptor (MOR)-TRPV1 crosstalk in TRPV1 activation involves morphine anti-nociception, tolerance and dependence

Yanju Bao\(^1,\#\), Yebo Gao\(^1,2,\#\), Liping Yang\(^3\), Xiangying Kong\(^4\), Jing Yu\(^5\), Wei Hou\(^1,*\), and Baojin Hua\(^1,*\)

\(\#\)These authors contributed equally to this work.

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Introduction

The \(\mu\)-opioid receptor (MOR) agonists are the class of analgesics most widely used to treat moderate and severe chronic pain. This class of analgesics includes morphine, the prototypical MOR agonist, which produces its analgesic effect at clinically relevant doses primarily through the G-protein coupled receptor MOR. It has been reported that most clinical opioids act on MOR. In particular, MOR expressed in the superficial dorsal horn of the spinal cord\(^1,2\) is essential for the analgesic effects of MOR agonists.\(^3,4\)

Chronic morphine administration inevitably results in the development of high tolerance. This propensity of morphine treated patients to develop tolerance, and the related loss of analgesic effectiveness, limits the use of morphine for chronic pain conditions. In addition, prolonged morphine use can lead to physical dependence, defined as a need for continuing drug use to prevent the symptoms of withdrawal. Although the mechanisms underlying morphine tolerance are not fully understood, many studies have reported that repeated morphine exposure opposes the analgesic effects of morphine by increasing the expression and release of chemokines, pro-inflammatory cytokines,\(^5\) and pronociceptive neurotransmitters in the spinal cord\(^6\) and DRG.\(^7\) It has been reported that sustained morphine administration results in numerous pronociceptive changes, including increased capsaicin evoked release and elevated concentrations of pronociceptive neurotransmitters within the spinal dorsal horn.\(^8,9\) A prominent feature of opioid-induced hyperalgesia is enhanced responsiveness to noxious thermal stimulation, suggesting that transient receptor potential vanilloid type 1 (TRPV1) channels may be an important element of this response.\(^10\)

TRPV1 is a nonselective cation (\(\text{Ca}^{2+}\)) channel that is involved in a variety of nociceptive processes\(^11\) and can be activated by multiple stimuli, including acidic pH (\(\leq 5.9\)), noxious heat (>42°C), endocannabinoids, endogenous lipids, and capsaicin.\(^12-15\) TRPV1 is widely distributed in the sensory terminals of
central and peripheral neurons. The effects of TRPV1 on thermal hyperalgesia and mechanical allodynia have been demonstrated in various diseases. Additionally, TRPV1 receptors are present in regions of the brain that regulate the transmission and modulation of pain. Chronic morphine administration increases TRPV1 expression in the spinal cord, DRGs, and sciatic nerve. In morphine resistant bone cancer pain, TRPV1 receptors are up-regulated in DRG neurons. In these patients, morphine may induce the expression of the TRPV1 receptor through the activation of the mitogen-activated protein kinase signaling pathway, including up-stream TRPV1 regulators. Similarly, the destruction of TRPV1 receptor-expressing sensory neurons by resiniferatoxin, an ultrapotent capsaicin analog, blocked morphine tolerance. In accordance with the results of previous studies, a recent report indicated blocking TRPV1 receptors with capsaicin (2.5 mg kg$^{-1}$) inhibited morphine tolerance induced by 5 days of morphine treatment. The results of these studies suggest that sustained opioid exposure also enhances TRPV1 receptor function in the periphery and plays an additional and essential role in sustained morphine induced thermal and tactile hypersensitivity.

Activation of MOR leads to dissociation of the inhibitory $G_{i/o}$-protein complex into $G_{i/o}$- and $G_{b/y}$-subunits, which then have an important impact on downstream signaling pathways. Opioids reduce adenyl cyclase (AC) activity through $G_{i/o}$-subunits. AC catalyzes adenosine triphosphate (ATP) conversion to cyclic adenosine monophosphate (cAMP), and this modulates the activation of protein kinase A (PKA) or cyclic nucleotide-gated ion channels. Morphine induces expression of the TRPV1 receptor via TRPV1 up-stream regulator activation in the mitogen-activated protein kinase (MAPK) signaling pathway. Current evidence emphasizes the importance of TRPV1 in morphine tolerance, dependence and morphine-induced antinociception.

Here, we review the current knowledge concerning these phenomena, focusing on morphine-induced TRPV1 activation. Furthermore, we highlight evidence characterizing downstream TRPV1 signaling molecules and their role in morphine tolerance, dependence and morphine-induced antinociception (Fig. 1).

**TRPV1 localization and function**

TRPV1 is predominantly expressed in unmyelinated neurons and in both the central and peripheral sensory terminals of primary sensory neurons. In the central nervous system (CNS), TRPV1 is located in regions that modulate nociception and control autonomic functions. TRPV1 receptor is an important integrator of various types of pain stimuli \textit{in vivo}. Therefore, the properties of polymodal nociceptors might be explained by TRPV1 responsiveness to various noxious stimuli. Chimeric and site-directed mutation studies of the TRPV1 channel have demonstrated that the phosphorylation of unique amino acid sites regulates the response of the channel to various stimuli, including capsaicin. Correspondingly, TRPV1 dephosphorylation can cause pharmacological desensitization of the channel.

TRPV1 appears to be critical for the transmission of noxious stimuli by nociceptive peripheral neurons and the development of inflammatory hyperalgesia. Accordingly, axonal transport of TRPV1 mRNA and TRPV1 protein expression are significantly increased in inflamed tissues. TRPV1 knockdown mice develop less thermal hyperalgesia and TRPV1 antagonists reverse inflammatory thermal hyperalgesia. Various factors modulate the response of TRPV1 to inflammatory stimuli, including growth factors, neurotransmitters, peptides or small proteins, lipids, chemokines and cytokines. TRPV1 receptor activation by chemokines and cytokines may lead to nociceptive effects that reverse the antinociceptive effects of morphine. Furthermore, while capsaicin treatment inhibits morphine induced antinociception in rats, capsaicin-induced thermal alldynia is alleviated by MOR activation in the central and peripheral nervous systems of rhesus monkeys.

Recently, a significant increase in TRPV1 immunoreactivity was demonstrated in the spinal cords, dorsal root ganglion (DRG) neurons and sciatic nerves of morphine-tolerant rats given daily intraperitoneal injections of 10 mg/kg morphine. Tolerance to morphine and tolerance-induced thermal hyperalgesia in the rats was suppressed by a 30 µg dose of the selective TRPV1 antagonist SB366791. Additionally, Niiyama et al. reported data indicating that TRPV1 antagonists acutely enhance morphine analgesia. In agreement with previous data from studies investigating rats, Nguyen’s research on mice also indicated that TRPV1 antagonists effectively prevent the development of morphine tolerance. Furthermore, our data first demonstrated that TRPV1 antagonists significantly reduced withdrawal symptoms in morphine-dependent mice.

**MOR and TRPV1 interaction**

TRPV1 and MOR co-localize in DRG neurons and the spinal cord. TRPV1 can be both sensitized and upregulated during inflammation and plays an essential role in the development of inflammation associated thermal hyperalgesia. A substantial increase in TRPV1 and MOR positive DRG neurons is caused by induced paw inflammation. Jeannette Endres-Becker et al. demonstrated that TRPV1 activity could be regulated by MOR ligands. TRPV1’s contribution to inflammatory hyperalgesia has been established through observations indicating that TRPV1 antagonists dose-dependently reverse both thermal and mechanical inflammatory hyperalgesia. In addition, thermal inflammatory hyperalgesia is significantly reduced in TRPV1 knockout mice. Jeannette Endres-Becker et al. also found a significant morphine-induced decrease in capsaicin-mediated TRPV1 activity in DRG neurons from complete Freund’s adjuvant treated animals. Therefore, it has been well established that TRPV1 expression plays an important role in the development of inflammation-induced hyperalgesia. Inflammation and morphine-induced hypersensitivity share many common characteristics, including hyperalgesia, alldynia and similar pronociceptive neuroadptive changes.

MOR are presynaptically expressed on the terminals of primary afferent neurons and on the postsynaptic neurons in
the dorsal horn of the spinal cord. The primary afferent neurons and the spinal cord participate in pain transmission and modulation and are the primary sites for the analgesic activity of MOR agonists. Treatment of adult rats with the capsaicin analog resiniferatoxin (RTX), a potent TRPV1 agonist, destroys TRPV1-expressing DRG and impairs thermal nociception in adult rats. Although RTX induces a significant reduction in presynaptic MOR, it potentiates and prolongs the analgesic effect produced by systemic or intrathecal injection of MOR agonists, including morphine and [D-Ala², N-Me-Phe⁴, Gly-ol⁵]-enkephalin (DAMGO). Additionally, TRPV1 antagonists have been demonstrated to decrease mechanical nociception in acute and chronic pain models. The reduction of TRPV1-expressing sensory neurons induced by RTX attenuates the development of morphine analgesic tolerance and alters the presynaptic effects of the MOR agonist in the spinal cord. Furthermore, the thermal hyperalgesia and mechanical allodynia that are normally induced by chronic morphine administration were absent in mice lacking TRPV1 expression. These effects were also opposed by treatment with the TRPV1 antagonist AMG0347 (3 mg/kg). These results suggest that TRPV1 channels are involved in the development of the thermal hypersensitivity associated with tissue inflammation. MOR and TRPV1 expression in primary afferent neurons and the activity of TRPV1 in DRG neurons can be inhibited by MOR. This inhibition is increased after thermal and mechanical inflammation. Furthermore, the effect of presynaptic MOR on TRPV1-expressing sensory neurons is particularly sensitive to down-regulation by μ opioid agonists during opioid tolerance development.

**MOR Sensitizes TRPV1 via β-Arrestin2**

The scaffolding protein PKA-anchoring protein 150 (AKAP150) mediates TRPV1 phosphorylation by protein kinases A and C. A role for anchoring and scaffolding proteins in the mediation of efficient downstream signaling cascades by organizing specific proteins and enzymes near their respective substrates has been established. β-arrestins, regarded as critical scaffold proteins, can form scaffolding complexes with a wide variety of proteins to regulate the strength and duration of diverse signaling pathways, such as Src kinase and phosphodiesterase 4D (PDE4D). Of particular interest, β-arrestins selectively associate with PDE4D to locally modulate subcellular cyclic AMP availability and subsequently activate PKA. Other research has presented evidence that β-arrestins contribute to ligand-activated β2-adrenergic receptors by scaffolding PDE4D isoforms that hydrolyze cAMP to regulate PKA activity and consequent receptor sensitivity.

β-arrestin molecules were originally identified as important mediators of metabotropic receptor desensitization that govern internalization of G-protein coupled receptors (GPCRs) following agonist exposure. Extensive research by numerous groups has revealed the contributions of β-arrestins to multiple physiological functions and processes. Recent research has demonstrated that β-arrestins are novel regulators that can regulate the function of several TRP channels and desensitize ionotropic receptors. Por ED et al. provided the first evidence that β-arrestin2 regulates TRPV1 receptor through its role as a scaffolding protein. Regulation of TRPV1 by β-arrestin2 induces PKA phosphorylation and effectively desensitizes the ionotropic...
receptor, which is implicated in a variety of inflammatory and pain conditions. Por ED et al. also reported that β-arrestin2 inhibited TRPV1-induced PKA phosphorylation, consequentially reducing receptor response to agonist-mediated stimulation. 

β-arrestin2 desensitizes TRPV1 in sensory neurons. MOR agonists, such as morphine and DAMGO, sequester β-arrestin2, reduce TRPV1/β-arrestin2 interactions and increase TRPV1 activity in peripheral sensory neurons. Previous studies showed that endogenous β-arrestin2 is required for the development of morphine tolerance, and mice lacking β-arrestin2 demonstrate increased sensitivity to the antinociceptive effects of morphine. Furthermore, hyperalgesia may rebound due to overactive PKA and result in phosphorylation and sensitization of TRPV1 during the process of patients ceasing opioid therapy. Hence, Rowan et al. speculate that the development of opioid-induced hyperalgesia (OIH) is due to chronic ligand treatment recruiting β-arrestin2 away from TRPV1 in sensory neurons.

cAMP/PKA

The MOR agonist morphine has well documented anti-inflammatory effects when injected directly into inflamed tissues in both animal models and human studies. Activation of TRPV1 by capsaicin induces hyperalgesia that can be inhibited by peripherally applied MOR agonists, such as morphine. Opioid withdrawal symptoms are associated with cAMP activity and increased concentrations of AC, PKA and the transcription factor cAMP response element binding protein. Phosphorylation mediated by cAMP/PKA can both sensitize TRPV1 and protect it from desensitization. Vetter I et al. reported that the anti-inflammatory action of peripheral opioids is mediated by the interaction of MOR and PKA-sensitized TRPV1. Phosphorylation of TRPV1 by numerous kinases, such as cAMP-dependent PKA, can regulate the function of the receptor. cAMP levels are elevated in inflamed tissues and the cAMP/PKA pathway appears to be important for inflammatory nociception. Thus, the cAMP/PKA pathway leads to the development of inflammatory hyperalgesia induced by pro-inflammatory regulators, including prostaglandin E2 (PGE2). In a variety of cell systems, including DRG neurons, accumulating evidence indicates that TRPV1 is modulated by PKA. For instance, activated PKA increases TRPV1 phosphorylation and channel sensitivity. Furthermore, PKA-mediated phosphorylation was demonstrated both to contribute to thermal-activated TRPV1 currents and to counteract Ca2+-dependent desensitization.

Previous experiments have shown that stimulation with capsaicin can reduce TRPV1 phosphorylation, and that PKA can rephosphorylate and subsequently re-sensitize TRPV1. Law et al. reported that MOR-mediated a decrease in intracellular cAMP levels, controlling PKA activity and decreasing TRPV1 channel activity. In accordance with this conclusion, pretreatment of DRG neurons with the potent cell-permeable cAMP analog 8-Br-cAMP reversed opioid mediated inhibition at TRPV1. Thus, opioid induced modulation of TRPV1 responses may occur in inflamed tissues where cAMP levels are elevated through inhibition of AC and the subsequent inhibition of TRPV1 responses via Gpi/o proteins by cAMP-dependent PKA. TRPV1 can also be inhibited via MOR-mediated inhibition of AC activity and decreased cAMP levels. Morphine can inhibit capsaicin responses when the cAMP pathway is activated. This occurs through opioid-modulation of adenylate cyclase and, therefore, indirectly through PKA-mediated TRPV1 sensitization. As TRPV1 is expressed peripherally and PKA-mediated sensitization occurs in these peripheral nociceptors via inflammatory mediators, non-central opioid receptor targeting under inflammatory conditions may prevent peripheral sensitization and contribute to analgesia.

MAPK

The MAPK family transduces a diverse group of extracellular stimuli into a wide variety of intracellular responses by inducing transcriptional, translational and post-translational modifications of target proteins. The MAPK family includes extracellular signal-regulated protein kinase (ERK), P38-mitogen activated protein kinase (P38 MAPK) and c-Jun N-terminal kinase (JNK). MAPK is a key regulator of cell proliferation, differentiation, survival, learning and memory, and evidence indicates that MAPK may be a key factor in pain hypersensitivity. It has been reported that treatment with multiple MAPK inhibitors reduces inflammatory and neuropathic pain without affecting the subject’s perception of basal pain. The involvement of similar cellular and molecular mechanisms has been demonstrated in the development of morphine tolerance and pathological pain. Because the same treatments that block pathological pain also attenuate opioid tolerance, the abnormal pain associated with the prolonged opioid usage is a key element affecting the behavioral symptoms characteristic of opioid tolerance.

In DRG neurons, chronic morphine administration leads to increased p38, ERK and JNK phosphorylation. Intrathecal selective MAPK inhibitor injections inhibit MAPK phosphorylation in DRG neurons by opposing p38, ERK and JNK phosphorylation. Chronic morphine administration has been reported to activate MAPK and lead to morphine tolerance. Hyperalgesia associated TRPV1 is considered a target of this mechanism.

ERK/MAPK

Significant recent advances indicate that morphine modulates ERK phosphorylation in cultured neuronal cells and in vivo. Morphine induced ERK activation was first described in recombinant Chinese hamster ovary (CHO) cells that were stably transfected with MOR. ERK activation was observed at 4 minutes and then, after 8 minutes, activation levels gradually decreased, recovering to basal activity levels after one hour of morphine treatment. In C6 glioma cells stably expressing MOR and COS-7 cells transiently transfected with MOR, the ERK cascade can be strongly activated by the application of the MOR agonist DAMGO. Human neuroblastoma SK-N-SH cells endogenously express MOR, and rapid ERK phosphorylation was
observed in these cells after acute morphine incubation. It is particularly interesting that prolonged morphine usage attenuated ERK phosphorylation. Accordingly, following either acute or chronic morphine incubation, MOR activation modulated ERK activity. It was demonstrated in vivo that long-term morphine administration in mice caused p-ERK elevation in the frontal cortex, hippocampus and striatum. In contrast, chronic morphine treatment led to decreased p-ERK levels in various tissues, including mouse and rat nucleus accumbens, mouse central amygdala and the cerebral cortex, median eminence and hypothalamic nuclei of humans and rats.

Additionally, morphine withdrawal was attenuated and withdrawal-induced allodynia was decreased after antisense oligonucleotide knockdown of spinal ERK and phosphorylation reduction using intrathecal MAPK kinase inhibitor U0126. Phosphatidylinositol 3-kinase (PI3K) and ERK can be activated in DRG neurons by intradermal injections of capsaicin and nerve growth factor (NGF). In primary sensory neurons, PI3K acts through TRPV1 sensitization to activate ERK and mediate inflammatory heat hyperalgesia. In light of these findings, it seems likely that PI3K-induced heat hyperalgesia is regulated by TRPV1 activity in an extracellular ERK-dependent manner.

P38 and JNK

Compared with ERK, fewer studies investigating the roles of p38 and JNK in morphine-induced tolerance and dependence at the supra spinal level have been conducted. It has been suggested that NGF increases TRPV1 in inflamed skin and DRG neurons through MAPK activation. Intrathecal administration of SB203580, a p38 specific inhibitor, significantly attenuated morphine analgesia tolerance. Additionally, it has been reported that p-p38 immunoreactive cells increased significantly in rats receiving intrathecal administration of 15 μg morphine.

Few studies have reported the role of JNK in morphine-induced anti-nociception and tolerance. c-Jun is downstream of JNK. It has been reported that during morphine withdrawal, increasing c-Jun levels affect some of the morphine withdrawal symptoms in the rat locus coeruleus cortex. Additionally, 9 days of subcutaneous morphine injections (10 mg/kg) resulted in elevated levels of the JNK family member JNK3 in the rat frontal cortex. However, this treatment did not result in increased JNK3 in the thalamus, hippocampus or locus coeruleus. Accumulating in vivo and in vitro evidence indicates that increases in phosphorylated JNK (p-JNK) are induced by repeated morphine treatment in rat DRG neurons.

SP and CGRP

Recent evidence suggests that long-term morphine exposure may contribute to morphine induced tolerance and dependence by regulating downstream targets of TRPV1, such as SP and CGRP. A combination of increased SP and CGRP expression in the sensory primary afferents and increased capsaicin-evoked release of SP and CGRP in the spinal dorsal horn have been described in both inflammation and morphine induced hyperalgesia. Chronic morphine exposure also causes physical dependence that manifests as withdrawal symptoms. SP and CGRP may influence morphine withdrawal symptoms, and high levels of SP and CGRP have been reported in animals exhibiting opioid withdrawal symptoms. Acute intrathecal treatment with SP or CGRP antagonists attenuates morphine withdrawal symptoms. CGRP-deficient mice show reduced withdrawal-associated jumping and SP knockout mice have decreased morphine reward and withdrawal. TRPV1 receptors co-localize with substance P (SP) and Calcitonin gene-related peptide (CGRP) in the primary sensory neurons, spinal cord, and DRG neurons. Capsaicin induces the release of SP and CGRP, whereas a TRPV1 antagonist capsazepine reverses these activities. Capsaicin causes SP and CGRP releases, whereas capsazepine reverses these activities. Activation of TRPV1 has been demonstrated to induce glutamate release, and glutamatergic synaptic transmission can be inhibited in rats by the TRPV1 antagonist SB366791 in the spinal dorsal horn after peripheral inflammation. Additionally, TRPV1 activation induces the central and peripheral endings of neurons associated with the neurogenic inflammatory response and nociceptive transmission to release peptide neurotransmitters, including CGRP and SP. However, it has been reported that capsaicin-induced SP release is reduced by opioids. Additionally, it has been reported that opioids inhibit neurogenic inflammation by reducing SP released from peripheral afferent terminals. Prolonged morphine usage enhances the release of CGRP induced by capsaicin. Neurotransmitter modulation through chronic opioid exposure activates TRPV1 and causes opioid associated tolerance and thermal hyperalgesia. In vitro and in vivo experiments in DRG neurons suggest that chronic morphine induced increases in CGRP and SP are the result of increased MAPK and cAMP response element-binding protein (CREB) phosphorylation. Chronic morphine exposure also provokes the manifestation of physical dependence and the resultant withdrawal symptoms. Extensive evidence indicates that neuropeptides may play an essential role in the manifestation of morphine withdrawal symptoms. Animals with opioid withdrawal symptoms exhibit elevation of both the neuropeptides SP and CGRP. Furthermore, acute intrathecal administration of SP or CGRP antagonists reduces the symptoms of morphine withdrawal. SP knockout mice exhibit diminished morphine reward and withdrawal responses, and CGRP-knockdown mice exhibit reduced withdrawal-induced jumping. Therefore, systemic TRPV1 receptors blockade by capsazepine may reduce withdrawal symptoms by preventing SP and CGRP releases in morphine-dependent mice. Chronic morphine activates TRPV1. In turn, TRPV1 modulates neurotransmitters such as glutamate, CGRP, and SP, and contributes to morphine tolerance and the associated thermal hyperalgesia.

Conclusions

Morphine is primarily used to treat patients experiencing moderate or severe pain. Unfortunately, the adverse effects of
The cessation of treatment.3,4,152 morphine dependence and exhibit withdrawal symptoms after tory animals and human patients. Subjects also develop physical developing rapidly in response to chronic morphine usage in labora-

tion of analgesic tolerance and physical dependence, morphine limit its use. These detrimental effects, which include in the central and peripheral nervous system is partly responsible for morphine-induced anti-nociception, and the development of morphine tolerance and dependence. TRPV1 is crucial for the transduction of noxious chemical and thermal stimuli and its activity can be modulated by numerous mediators, including growth factors, neurotransmitters, peptides, small proteins, lip-

ratory signal-regulated protein kinase (ERK)-dependent manner. These studies imply that TRPV1 is not solely a thermo-

receptor; instead, its activity appears to be modulated by various molecules that act through distinct pathways. A number of

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