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The Role of Mitogen-Activated Protein Kinase (MAPK) in Morphine Tolerance and Dependence

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Abstract Despite the existence of a large body of information on the subject, the mechanisms of morphine tolerance and dependence are not yet fully understood. There is substantial evidence indicating that mitogen-activated protein kinase (MAPK), a family including extracellular signal-regulated protein kinase, p38 MAPK, and c-Jun N-terminal kinase, can be activated by chronic morphine treatment in the central and peripheral nervous systems and that application of a MAPK inhibitor reduces morphine tolerance and dependence. While the exact mechanism is not completely understood, recent evidence suggests that the activation of MAPK induced by long-term morphine exposure may participate in tolerance and dependence by regulating the downstream targets, such as calcitonin gene-related peptide, substance P, nitric oxide, transient receptor potential vanilloid 1, and proinflammatory cytokines. In this review, we focus on the current understanding of the role of MAPK signaling pathways in morphine tolerance and dependence.

Keywords Morphine · Tolerance · Dependence · Mitogen-activated protein kinase · Activation · Downstream target

Introduction

Morphine, a strong opioid acting at the mu opioid receptor (MOR), is one of the most commonly used drugs in the treatment of severe and chronic pain. Besides the known side effects such as respiratory depression, nausea, vomiting, cough suppression, and sedation, its use for chronic pain conditions is limited by the development of tolerance, a loss of analgesic effectiveness of the drug during repeated use. In addition, prolonged use of morphine can also lead to physical dependence, a need for continuing use of the drug to prevent the symptoms of withdrawal. As such, the possible mechanisms for these phenomena have been intensively studied in an attempt to understand and prevent them. For decades, research has focused on the possible role of MOR desensitization and internalization, the coupling of MOR to alternative G proteins and antiopioid systems in tolerance and dependence [1]. Despite considerable progress, the molecular and cellular mechanisms underlying morphine tolerance and dependence are not yet completely understood.

Intracellular signaling cascades are the main routes of communication between the plasma membrane and regulatory targets in various intracellular compartments. Sequential activation of kinases is a common mechanism of signal transduction in many cellular processes. During the past two decades, several related intracellular signaling cascades have been described, which are collectively known as mitogen-activated protein kinase (MAPK) signaling cascades. There are four major groups of MAPK: extracellular signal-regulated protein kinase (ERK), p38 MAPK, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated protein kinase 5 (ERK5) that represent the different signaling cascades and transduce a broad range of extracellular stimuli into diverse intracellular responses by both transcriptional and nontranscriptional regulation [2, 3]. MAPK has been classically regarded as a regulator of cell proliferation, differentiation, survival, learning, and memory and is now also appreciated to have an important role in regulating pain hypersensitivity. Inhibition of MAPK has been shown to attenuate inflammatory and neuropathic pain without changing basal pain perception [4–8].

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Morphine tolerance and pathological pain are predicted to involve similar cellular and molecular mechanisms [9, 10]. Over the last decade, compelling evidence has accumulated indicating that MAPK plays an important role in morphine-induced tolerance and dependence via both neuronal and glial mechanisms. In this review, we summarize the current understanding of these phenomena, with a focus on the activation of MAPK induced by chronic morphine and the effect of inhibition of MAPK on morphine tolerance and dependence. We then highlight evidence regarding the downstream signaling molecules of MAPK in tolerance and dependence (Fig. 1).

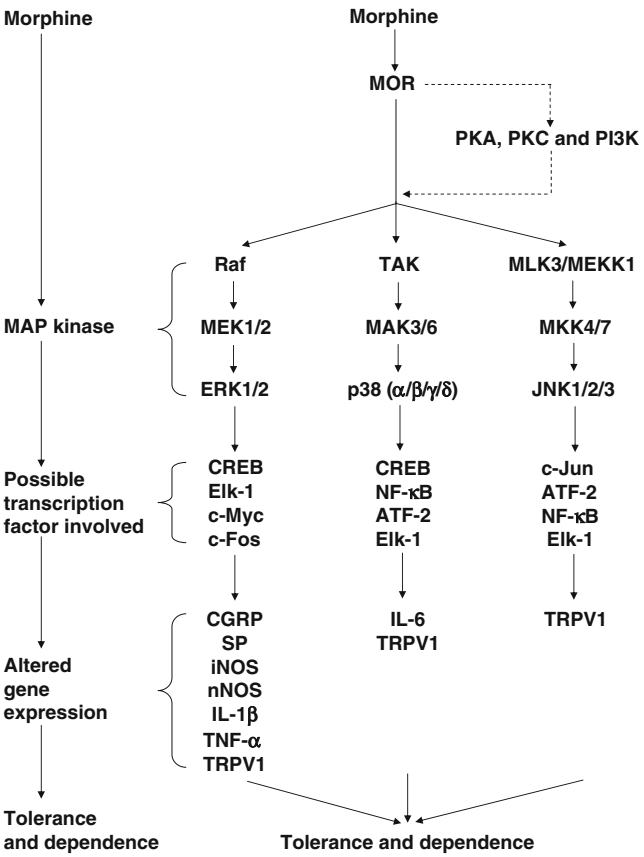

Fig. 1 Simplified scheme of signal transduction in morphine tolerance and dependence. By acting on MOR, chronic administration of morphine leading to tolerance and dependence activates MAPK, including ERK, p38, and JNK, possibly via protein kinase A (*PKA*), protein kinase C (*PKC*), and phosphatidylinositol 3-kinase (*PI3K*). The nuclear translocation of phosphorylated MAPK results in the phosphorylation of their transcription factors, such as cAMP-regulatory element-binding protein (*CREB*), Ets-like protein-1 (*Elk-1*), c-Myc, c-Fos, nuclear factor-κB (*NF-κB*), activating transcription factor-2 (*ATF-2*), and c-Jun. These events lead to enhanced expression of downstream effectors such as CGRP, SP, iNOS, nNOS, IL-1β, TNF-α, IL-6, and TRPV1, which contribute to morphine tolerance and dependence. ERK5, the fourth member of the MAPK family, is not included here because there is lack of information on ERK5 in morphine tolerance and dependence

Activation of MAPK in Morphine Tolerance and Dependence

It is well-established that upon repeated exposure to opioids, profound long-lasting adaptation occurs in discrete nervous circuits [11]. Emerging evidence suggests that MAPK activation leads to adaptive changes, which have been postulated to be involved in the development of morphine tolerance and dependence.

Morphine-induced activation of ERK was first described in cells expressing the transfected MOR. Marked activation of ERK in recombinant Chinese hamster ovary (CHO) cells stably transfected with MOR was observed at 4 min, gradually declined after 8 min, and returned to near basal activity after 1 h of morphine incubation [12]. Single application of a MOR agonist [D-Ala2, N-Me-Phe4, Gly5-ol]-enkephalin also strongly activated the ERK cascade in C6 glioma cells expressing MOR, and similar activation was seen in transiently transfected COS-7 cells [13]. With acute morphine treatment, a rapid phosphorylation of ERK was found within a few minutes in human neuroblastoma SK-N-SH [14] and SH-SY5Y cells [15, 16], which endogenously express MOR. Interestingly, prolonged morphine treatment (up to 3 days) decreased the level of phosphorylated ERK in SH-SY5Y cells, which was profoundly augmented during morphine withdrawal, providing evidence that MOR activation differentially modulates ERK activity after acute and chronic morphine treatment as well as during morphine withdrawal in SH-SY5Y cells [16]. Taken together, these studies as above indicate that phosphorylation of ERK can be modulated by morphine in both cultured cells of non-neuronal origin and neuronal origin. In vivo, activation of ERK has been demonstrated in brain regions, which are thought to play an important role in tolerance and dependence, following repeated morphine administration in rodents. For example, both single and repeated subcutaneous (s.c.) injection of morphine (5 and 10 mg/kg, respectively) increased ERK activity in the nucleus accumbens in mice [17, 18]. Chronic morphine treatment via s.c. implantation of one pellet (containing 75 mg of morphine base) and acute intraperitoneal (i.p.) injection of morphine at a dose of 10 mg/kg was reported to increase phosphorylated ERK (p-ERK) levels without increasing overall levels of ERK in the rat [19] and mouse [20] ventral tegmental area, respectively, a brain region involved in the rewarding properties of morphine, which in turn contributes to drug-induced increases in other neuro-adaptive changes. In the mouse pons/medulla, 7 days of repeated morphine injection at a dose of 10 mg/kg remarkably enhanced the p-ERK immunoreactivity [21]. Elevated p-ERK level was detected in the locus coeruleus and caudate/putamen of morphine-tolerant rats implanted subcutaneously with 75 mg of morphine pellet [22]. Both acute (10 or 100 mg/kg; s.c.) and chronic intermittent morphine (10–40 mg/kg; s.c.) caused ERK activation in the mouse anterior cingulate,

somatosensory, and association cortices as well as the locus coeruleus [23]. Naloxone-precipitated withdrawal after chronic morphine has also been shown to activate ERK in the rat locus coeruleus, the nucleus of the solitary tract, and the arcuate hypothalamic nucleus [24]. Recently, Li et al. [25] demonstrated that long-term morphine treatment (10 mg/kg; s.c.) increased p-ERK in the mouse frontal cortex, hippocampus, and striatum. However, a decrease in p-ERK level caused by chronic morphine was observed in the mouse [23] and rat [26] nucleus accumbens, mouse central amygdala [23], and also in the human or rat cerebral cortex, median eminence, and hypothalamic nuclei [15, 24]. These results suggest that morphine-induced ERK activation might have brain region-specific properties.

Accumulating evidence has suggested that the spinal cord is a crucial site in mediating opioid dependence [27–29]. Recently, much attention has focused on the role of ERK in morphine tolerance and dependence at the spinal cord level in rats. For example, chronic morphine treatment with ascending doses (10–50 mg/kg, s.c.) induced the activation of ERK in spinal dorsal horn neurons, which was further increased after naloxone-precipitated withdrawal, indicating that activation of the spinal ERK signaling pathway contributes naloxone-precipitated withdrawal in morphine-dependent rats. In addition, this activation can be inhibited by nitric oxide synthase (NOS) inhibitors and *N*-methyl-D-aspartate (NMDA) receptor antagonist, suggesting that nitric oxide (NO) and NMDA receptor are implicated in activation of ERK during morphine dependence and withdrawal [30, 31]. In addition, a recent study showed that an increase in p-ERK level in the spinal cord was seen after intrathecal (i.t.) injection of morphine (15 μg/day) for 7 days in rats [32]. Different from Cao and colleagues' study, however, the increase of p-ERK was found preferentially in astrocytes but not in neurons. These discrepancies might be caused by injection route (s.c. vs i.t.), dose (ascending doses 10–50 mg vs 15 μg), and survival time (5 vs 7 days). We have demonstrated that phosphorylation of ERK in primary afferent neurons occurs in response to 7 days of i.p. injection of morphine at dose of 10 mg/kg in vivo [33], similar to the effect of chronic morphine on phosphorylation of ERK in cultured dorsal root ganglion (DRG) neurons [34]. Different from the changes observed in the spinal cord and DRG neurons of rats, surprisingly, long-term treatment of morphine (s.c. implantation of a 75-mg morphine pellet) did not change p-ERK level in the mouse plantar tissue [35].

Compared to many studies of ERK in morphine tolerance and dependence at the supraspinal level, information on p38 and JNK is scarce. Cui et al. [36] showed that the number of spinal phosphorylated p38 (p-p38) immunoreactive cells in rats began to increase significantly following 3 days of repeated i.t. administration of morphine at a dose of 15 μg and further enhanced from 5 to 7 days; however, p38 activation was not found in the animals treated with acute morphine. Double staining indicated that chronic morphine treatment only resulted in the phosphorylation of p38 in spinal microglia, but not in astrocytes or neurons. These data as above are confirmed by a recent study in which a significant increase of p-p38 was found in spinal microglia after long-term, but not acute, treatment of morphine in rats [32]. In addition, the selective neuronal NOS (nNOS) inhibitor 7-nitroindazole sodium salt (7-NINA) significantly inhibited the increase of p38 activation in the spinal microglia induced by repeated morphine treatment, suggesting that neuronal NO signals to microglia, leading to the upregulation of microglial p-p38 in morphine-tolerant animals [37]. Supporting the effects of chronic morphine on p38 in cultured rat DRG neurons [34], phosphorylation of p38 was detected in DRG neurons of morphine-tolerant rats given daily i.p. injection with a dose of 10 mg/kg [33]. It is worth noting that p-p38 was significantly decreased in plantar tissue of tolerant mice induced by s.c. implantation of a 75-mg morphine pellet [35]. These results suggest that different mechanisms may be implicated in the p38 response, like ERK, to morphine tolerance in peripheral tissue vs spinal glial cells and DRG neurons. Different from increased p-ERK in spinal astrocyte and neurons in morphine-tolerant rats, on the other hand, increased p-p38 was found in microglia, indicating that ERK and p38 might contribute to morphine tolerance via different cellular mechanisms. Indeed, a new finding reported by Wang et al. [32] demonstrated that inhibition of the activated ERK in astrocytes suppressed morphine-induced upregulation of interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) and blockade of activated p38 in microglia inhibited morphine-induced IL-6 upregulation.

During morphine withdrawal, c-Jun, downstream of JNK, was increased within rat locus coeruleus [38] and cortex [39], the sites believed to mediate some of the symptoms of morphine withdrawal. In addition, 9 days of morphine injection (10 mg/kg; s.c.) increased JNK3, one member of the JNK family, in rat frontal cortex but not in thalamus, hippocampus, and locus coeruleus at mRNA level [40]. Both in vivo and in vitro studies showed that repeated morphine treatment increases phosphorylated JNK (p-JNK) in rat DRG neurons [33, 34], but not in spinal cord [32].

ERK5, also known as big MAPK1, is twice the size of other MAPK [41, 42]. It has been recently shown that the ERK5 cascade contributes to pain hypersensitivity. Nerve injury increased phosphorylation of ERK5 in rat spinal microglia and DRG neurons, and antisense knockdown of ERK5 suppressed nerve injury-induced neuropathic pain and decreased microglial activation [43]. Injection of complete Freund's adjuvant into a rat hindpaw produced sustained ERK5 activation in DRG [44, 45] and spinal

dorsal horn neurons [45]. It has been proposed that morphine and pathological pain may share common cellular mechanisms [9, 10]. Furthermore, some of the functions attributed to ERK may actually be carried out by ERK5 because PD98059 and U0126, which were identified as ERK-specific inhibitors, also inhibit the ERK5 pathway [46, 47]. It might thus be interesting to explore the role of ERK5 in morphine tolerance and dependence.

Inhibition of MAPK in Morphine Tolerance and Dependence

Cao and colleagues [30, 31] showed that attenuation of spinal ERK phosphorylation by i.t. injection of an ERK inhibitor U0126 at a dose of 5 μg or knockdown of the spinal ERK by antisense oligonucleotides at a dose of 10 μg not only decreased the scores of morphine withdrawal, but also attenuated withdrawal-induced mechanical allodynia in rats. The involvement of spinal ERK in morphine withdrawal was further supported by the findings that i.t. application of inhibitors of the NMDA receptor, protein kinase C (PKC), and NOS not only suppressed withdrawal-induced ERK activation but also attenuated morphine withdrawal symptoms [30, 31]. Consistently, i.t. pretreatment with 10 μg of ERK inhibitor U0126 [33] or PD98059 [32] also decreased morphine tolerance in rats. In contrast, i.p. administration of an ERK inhibitor SL327 (50 mg/kg) prior to s.c. injection of morphine had no effect on the development of tolerance or withdrawal signs in mice [48]. These discrepancies may be related to the testing paradigms, animal species, doses, and route of administration. Studies using ERK-deficient mice [49] might be helpful to further elucidate the role of ERK in tolerance and dependence. Although it is well-established that repeated morphine administration regulates the activity of ERK in the brain, there is still lack of information on the functional significance of this phenomenon in tolerance and dependence, and the study in behavioral correlation of these anatomical findings is needed.

A few studies have directly linked the role of p38 activation in the spinal dorsal horn microglia and DRG neurons to morphine tolerance. Cui et al. [36] found that repeated, but not single, i.t. administration of SB203580 (2 or 10 μg), a specific p38 inhibitor, attenuated tolerance to morphine analgesia assessed by the tail flick test in rats, which was further confirmed by tail flick [32] and Hargreaves tests [33]. A recent study suggests that the inhibitor of microglial activation minocycline, at doses of 20, 50, and 100 μg, depressed morphine tolerance due to the inhibition of p38 activation in rat spinal microglia [50].

To date, little work has been performed on the effects of the inhibition of JNK on morphine tolerance and dependence. One study demonstrated that i.t. injection of SP600125 (50 μg), a selective JNK inhibitor, significantly reduced morphine tolerance in rats [33].

Downstream Targets of Activated MAPK in Morphine Tolerance and Dependence

MAPK transduce a broad range of extracellular stimuli into diverse intracellular responses by producing changes in transcription as well as by translational and posttranslational modification of target proteins. A noninclusive list of examples of substances involved in morphine tolerance or dependence includes: calcitonin gene-related peptide (CGRP), substance P (SP), NO, cytokines, cyclooxygenase, PKC, NMDA, and cholecystokinin [51, 52]. Does the MAPK signaling pathway act by regulating the production of some substances as above in tolerance and dependence?

CGRP- and SP-like immunoreactivity was dramatically increased in the spinal dorsal horn of animals receiving prolonged morphine administration [53–55]. I.t. application of CGRP and SP receptor antagonists inhibited the development of tolerance to this opioid, suggesting that activation of spinal CGRP and SP receptors contributes to morphine tolerance [32, 51, 53, 54]. Enhanced expression of CGRP and SP in the spinal dorsal horn most likely reflects an increased production of these two peptides in DRG neurons [34]. Previous study demonstrated that ERK might be involved in mediating the synthesis of neuropeptides [56]. Ma et al. [34] reported that chronic exposure of cultured DRG neurons in rats to morphine induced significant increase in the phosphorylation of ERK and in the expression of CGRP and SP, which can be blocked by the opioid receptor antagonist naloxone. Double immunostaining experiments showed that p-ERK was coexpressed with CGRP and SP in DRG neurons. Exposure to an ERK inhibitor, PD98059, blocked morphine-induced increase of p-ERK as well as enhanced CGRP and SP, indicating a role for p-ERK in the expression of CGRP and SP in morphine tolerance. Interestingly, it was recently found by the same group that the increased p-ERK localized in astrocytes induced by chronic morphine was blocked by i.t. injection of a CGRP receptor antagonist BIBN4096BS (8.69 μg) [32], suggesting that there is a cross talk between p-ERK and CGRP in morphine tolerance.

NO is generated from L-arginine by NOS, of which three forms have been identified: nNOS, inducible NOS (iNOS), and endothelial NOS [57]. Accumulating evidence shows that NO is responsible for morphine tolerance [10]. Attenuation of the spinal ERK phosphorylation by the ERK inhibitor U0126 (5 μg) inhibited the increase of nNOS and iNOS expression in the spinal cord of morphine withdrawal rats. On the other hand, i.t. pretreatment with either the nonselective NOS inhibitor *N*(*G*)-nitro-L-arginine methyl ester (400 μg), the nNOS inhibitor 7-nitroindazole

(400 μg), or the iNOS inhibitor aminoguanidine (150 μg) could reduce morphine withdrawal-induced increase of p-ERK expression in rat spinal cord [31]. These findings suggest that cross talk between NO and the ERK signaling pathway mediates morphine withdrawal. Similarly, morphine tolerance and activation of p38 MAPK in the spinal microglia in rats induced by chronic i.t. administration of morphine were simultaneously attenuated by i.t. application of 7-NINA (25 μg) [37]. Further studies are needed to test if there is also a feedback loop between NO and the p38 signaling pathway, like the relationship of NO and ERK, in morphine tolerance and dependence.

The transient receptor potential vanilloid 1 (TRPV1) receptor, which is a nonselective cation channel gated by noxious heat, protons, and capsaicin, plays a critical role in the development of thermal and mechanical hyperalgesia under pathological conditions [58–60]. Deletion of TRPV1-expressing afferent neurons by resiniferatoxin, an ultrapotent capsaicin analog, attenuated the development of the analgesic tolerance resulting from both i.t. and systemic injections of morphine in rats [61], suggesting a functional role of TRPV1 in morphine tolerance. A recent study showed a marked increase in TRPV1 immunoreactivity in spinal cord dorsal horn, DRG neurons, and sciatic nerve of morphine-tolerant rats given daily i.p. injection with a dose of 10 mg/kg. Antagonism of TRPV1 by the selective TRPV1 antagonist SB366791 at a dose of 30 μg suppressed the tolerance to morphine thermal analgesia and tolerance-induced thermal hyperalgesia [33]. Furthermore, both thermal and tactile hypersensitivity elicited by chronic morphine were absent in TRPV1 KO mice and reversed by oral administration of the TRPV1 antagonist AMG0347 (3 mg/kg) [62]. Importantly, inhibition of p38, ERK, or JNK activation by the selective MAPK inhibitors (10, 10, and 50 μg for p38, ERK, and JNK inhibitors, respectively) not only reduced morphine tolerance but also reduced the chronic morphine-induced increase in TRPV1 in the spinal cord, DRG neurons, and sciatic nerve, suggesting that the activation of MAPK induced by chronic morphine participates in morphine tolerance and associated hyperalgesia by regulating the downstream target TRPV1 [33]. In other words, the MAPK signaling pathway contains the upstream regulators of TRPV1, which contribute to the tolerance of morphine.

Proinflammatory cytokines like TNF-α, IL-1β, and IL-6 induce and facilitate neuropathic as well as inflammatory pain [63, 64]. Recent studies indicate that cytokines contribute to morphine tolerance and dependence at the spinal cord level [52]. For example, chronic morphine treatment activated spinal glial cells and upregulated proinflammatory cytokines in microglia. Morphine tolerance was slowed or reversed by the inhibition of spinal proinflammatory cytokines [52]. A link to ERK and p38 MAPK was reported by Wang et al. [32] who demonstrated that i.t. administration of morphine for 7 days significantly increased p-ERK in spinal cord microglia and p-p38 in astrocytes in rats. Inhibition of the ERK pathway suppressed the development of tolerance and morphine-induced upregulation of IL-1β and TNF-α. Blockade of p38 activity also inhibited the development of tolerance and morphine-induced IL-6 upregulation. Taken together, these data suggest that chronic morphine induces the activation of ERK and p38, leading to increased synthesis and release of proinflammatory mediators resulting in tolerance to morphine-induced analgesia.

Summary

In summary, a series of findings from different laboratories indicate that the activation of MAPK in the central and peripheral nervous system contributes to morphine tolerance and dependence. Several downstream pathways are involved in this process. MAPK and its downstream molecules may, therefore, be potential targets for reducing morphine tolerance and dependence. Although the phenomena of MAPK in morphine tolerance and dependence have been widely studied, many questions remain. Morphine analgesic is one of the primary treatments for pain management in cancer patients reporting moderate to severe pain and is being increasingly used for noncancer chronic pain [65, 66]. Further studies are needed to investigate whether the coadministration of MAPK inhibitor with morphine can improve morphine analgesic efficacy and safety in cancer and noncancer chronic pain and allow reductions in morphine dose. While tolerance and dependence may develop with the repeated use of opioids, such as morphine, fentanyl, codeine, and methadone, the molecular mechanisms that mediate tolerance and dependence in each of these drugs might be different [67, 68]. Compared to many studies of MAPK in tolerance and dependence induced by morphine, information on other opioid analgesics is scarce. Despite increasing evidence supporting an important role of MAPK in the regulation of morphine tolerance and dependence, the upstream and downstream mechanisms of MAPK activation are still elusive. In particular, the key molecular targets that mediate the action of MAPK in the modulation of tolerance and dependence remain to be identified.

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