

The Roles of Excitatory Amino Acids and Cytokines in Morphine Tolerance: Effect of Tricyclic Antidepressant Amitriptyline

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Abstract: Morphine is an effective analgesic in clinical practice; however, its long-term administration causes tolerance, thereby limiting its use. The development of opioid tolerance and its associated hyperalgesia has been associated with interactions between opioid receptors and excitatory amino acids or cytokines. Targeted inhibition of excitatory amino acid- and cytokine-mediated signaling pathways may allow development of novel therapeutic strategies for treating opioid tolerance. Recent studies showed that administration of amitriptyline (a tricyclic antidepressant widely used in the treatment of neuropathic pain) attenuated morphine tolerance and preserved the antinociceptive effect of morphine. This ability might be related to the effects of tricyclic antidepressants on pro-inflammatory cytokine release and glutamate transporter expression in dorsal horn during morphine tolerance. Here, we will review evidence for the role of excitatory amino acids and cytokines in the development of morphine tolerance and discuss potential mechanisms by which tricyclic antidepressants attenuate morphine tolerance.

INTRODUCTION

Morphine is one of the most potent analgesic drugs used in clinical pain management. It is often used for long-term pain management in patients with cancer pain and neuropathic pain. However, its use for treatment of chronic pain is limited by side effects, especially the development of morphine tolerance (that is, a loss of analgesic effectiveness) [1]. Morphine tolerance is a complex physiological response that involves a within-system and a between-system adaptation [2]. The within-system adaptations, which include opioid receptors uncoupling from G-proteins and receptor down-regulation, are well-known mechanisms of opioid tolerance [3, 4]. Recent studies have proposed that between-system adaptations, such as the pain facilitatory systems (opiate-activated opponent systems) also may play an important role in the development of opioid tolerance. Excitatory amino acids (EAAs), their activated receptors [e.g., N-methyl-D-aspartate (NMDA) receptors], and the subsequent downstream signals (e.g., nitric oxide) are probably involved in a between-system mechanism of opioid tolerance [5]. In addition, pro-inflammatory cytokines released from activated glial cells after repeated morphine injections have been reported to participate in this between-system mechanism [6].

Tricyclic antidepressants are widely used to treat chronic pain such as neuropathic and inflammatory pain conditions in clinical [7, 8]. According to a recent Cochrane meta-analysis, which included 61 studies, tricyclic antidepressants are considered to be efficient for neuropathic pain relief on

the basis of sufficient class I trials [9]. Researches showed that tricyclic antidepressants amitriptyline possesses potent sodium channel blocking activity and a potent local anesthetic effect than bupivacaine [10-12]. Intrathecal administration of amitriptyline effectively attenuates pain and thermal hyperalgesia in rat inflammatory and neuropathic pain models, particularly when combined with opiates and clonidine [13-15]. Tricyclic antidepressants produce analgesia by various mechanisms involving NMDA receptors, biogenic amines, opioids, inflammatory mediators, and substance P [12, 16, 17]. New studies have revealed that intrathecal administration of the tricyclic antidepressants amitriptyline attenuates the development of morphine tolerance through targeted inhibition of EAA- and pro-inflammatory cytokine-mediated signaling pathways. Here, we will review evidence for the role of EAAs and cytokines in the development of morphine tolerance and discuss potential mechanisms by which amitriptyline might prevent morphine tolerance.

EXCITATORY AMINO ACIDS AND MORPHINE TOLERANCE: EFFECT OF AMITRIPTYLINE

EAAs such as glutamate and aspartate are the principal excitatory neurotransmitters in neuronal circuits; they are involved in a variety of central nervous system functions, including pain modulation [18]. Glutamate and aspartate have been shown to be involved in nociception transmission in the spinal cord [19, 20]. A large number of glutamate binding sites exist in the substantia gelatinosa of the rat spinal cord and correlate well with the projection areas of primary glutamatergic terminals [21]. Intrathecal injection of EAAs results in hyperalgesia and allodynia in rats [19]. Although four days of spinal morphine infusion in rats had little effect on the basal level of EAAs [22], post-treatment with naloxone evoked a reliable, time-dependent increase in dialysate glutamate and taurine concentration, but not other

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amino acids in chronic intrathecal morphine-infused rats [22]. No effect was seen in saline-infused rats.

Consistently, we have found that acute morphine treatment increases the levels of the dopamine metabolite DOPAC and glutamate in the striatum, nucleus accumbens, and locus coeruleus neurons in naloxone-precipitated morphine-tolerant rats [23]. Intrathecal morphine challenge induced the release of glutamate and aspartate in spinal cord of morphine-tolerant rats and produced a loss of morphine's analgesic effect [24]. Co-administration of morphine with the NMDA antagonist MK-801 not only attenuated morphine tolerance development, but also blocked morphine-induced spinal EAAs release [24-26]. In a clinical setting, increased glutamate and aspartate concentrations in the cerebral spinal fluid and a reduction in the analgesic effect of morphine were observed in terminal cancer pain patients who received long-term intrathecal morphine for pain relief [27]. These findings indicate a correlation between spinal cord EAA release and the development of morphine tolerance. Recently, amitriptyline has been shown to prevent the development of morphine tolerance [28, 29]. Moreover, pre- and post-treatment of amitriptyline attenuated the morphine-evoked EAA release in morphine-tolerant rats [28, 29]. These findings suggest that amitriptyline produced prevention of morphine tolerance might result from a reduction in spinal EAA release.

Glutamate mediates its actions through two types of receptor, metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (iGluRs). The mGluRs mediate slow glutamate response by coupling to a variety of signal transduction system via G proteins [30]. As a consequence, mGluR signaling plays an important role in the processing underlying synaptic plasticity [31]. Studies have shown that mGluR 5 antagonists inhibited inflammatory and neuropathic pain in rats [32, 33] and mice [34, 35]. In addition, acute and chronic administration of mGluR5, mGluR2/3 and mGluR7 antagonist also attenuates allodynia and hyperalgesia and potentiates morphine's efficacy in neuropathic pain mice [36]. The iGluRs, mediate fast excitatory glutamate response, are subdivided into NMDA, AMPA (α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) and kainite receptors. Activation of spinal iGluRs plays a crucial role in the development of tolerance to the analgesic effects of morphine [5, 37, 38]. The induction of EAA release by repeated morphine injections could activate glutamate receptors in the spinal cord. Co-administration of morphine with an NMDA receptor antagonist (MK-801) and NMDA receptor/glycine site antagonist (ACEA-1328) effectively prevents the development of morphine tolerance in several animal models, including rats, mice, and guinea pigs [39-43]. Furthermore, competitive NMDA receptor antagonist LY274614 prevent antinociceptive tolerance to the highly selective μ -opioid antagonist DAMGO [44]. The competitive AMPA receptor antagonist LY293558 attenuates and reverses the analgesic tolerance to morphine [37]. Deletion of the glutamate receptor 5 subunit of kainite receptor also prevents morphine tolerance [38]. Thus, it is clear that EAA-stimulated iGluRs receptor activation within the spinal cord is involved in the development of morphine tolerance. Amitriptyline and other tricyclic antidepressants have shown with high binding affinity to NMDA receptors [45], and function as NMDA antagonists [46, 47]. However,

the underlying mechanisms of amitriptyline mediated effects via regulating the glutamate receptor in morphine tolerance still need further investigation.

GLUTAMATE TRANSPORTER EXPRESSION AND MORPHINE TOLERANCE: EFFECT OF AMITRIPTYLINE

The termination of glutamatergic transmission and clearance of excess synaptic glutamate depend on high-capacity Na⁺-dependent glutamate transporters, including GLAST, GLT-1, and EAAC1. These three glutamate transporters are expressed at the highest density within the superficial dorsal horn of spinal cord in rats and mice, especially in the pain-related regions [48, 49]. GLT-1 and GLAST are exclusively distributed in glial cells at per-synaptic sites in the superficial dorsal horn [49]. EAAC1, in addition to being expressed in the spinal cord neurons, is detected in the dorsal root ganglion and distributed predominantly in the small dorsal root ganglion neurons (but not in dorsal root ganglion glial cells) [48]. Chronic morphine administration, given by either intrathecal bolus injections or continuous infusion, induces dose-dependent down-regulation of glutamate transporter protein expression in the spinal cord dorsal horn [50, 51]. Administration of glutamate transporter activators (e.g., MS-153 or riluzole) attenuates the development of morphine tolerance and associated thermal hyperalgesia [51, 52]. In contrast, disturbance of spinal glutamate transporter activity by the glutamate transporter inhibitor l-trans-pyrrolidine-2-4-dicarboxylate potentiates the development of both morphine tolerance and thermal hyperalgesia [51]. These results indicate that down-regulation of spinal glutamate transporters after repeated morphine injection might contribute to the development of morphine tolerance.

Interestingly, co-administration of amitriptyline with morphine has been shown to up-regulate the expression of glutamate transporters in chronically morphine-infused rats [53]. The NF- κ B pathway might be involved in this effect. NF- κ B is known to activate many genes in the response to inflammation and tissue injury. A consensus NF- κ B site is found in the 5' untranslated region of the GLT-1 gene [54]. NF- κ B-dependent GLT-1 expression is positively regulated by epidermal growth factor and negatively regulated by TNF- α . [54]. TNF- α recruits N-myc (transcription regulatory factor) to the glutamate transporter promoter, which leads to the conversion of NF- κ B to a transcriptional repressor and results in reduction of GLAST and GLT-1 expression [55, 56]. The serine/threonine kinase (Akt, also called protein kinase B) activates NF- κ B via I κ B phosphorylation, resulting in I κ B degradation and induction of GLT-1 (but not GLAST) expression [57]. Co-infusion of amitriptyline with morphine significantly increases phosphorylated I κ B α level, promotes I κ B α degradation, leads to translocation of the activated NF- κ B complex to its nuclear target, and results in up-regulation of glutamate transporter expression. Blockade of NF- κ B activation by Ro 1069920 completely prevented amitriptyline-induced GLAST and GLT-1 up-regulation and even further down-regulated the expression of all three glutamate transporters in chronically morphine-infused rats. These studies suggest that amitriptyline up-regulates glutamate transporter expression, at least in part, by enhancing NF- κ B transcription in the morphine-tolerant rats [53].

Protein kinase A (PKA) and protein kinase C (PKC) might also be involved in amitriptyline-induced up-regulation of surface glutamate transporter expression in the morphine tolerant animals. Activation of PKA and PKC regulates glutamate transporter trafficking between the cytoplasm and the plasma membrane of neurons and glial cells [58-61]. PKC activation decreases both the activity and cell surface expression of GLT-1 in a variety of systems [62-64]. This decrease might be caused by the formation of PKC α -GLT-1 complexes, which induce the PKC-dependent internalization of GLT-1 [59]. Interestingly, PKA inhibition reduces cell surface expression of EAAC1 and GLAST but increases the cell surface expression of GLT-1 [65]. In morphine-tolerant rats, acute pretreatment with the PKC inhibitor G δ 6805 increases GLT-1 cell surface expression and EAA uptake after morphine challenge [28], whereas acute treatment with PKA inhibitor H89 increases GLAST expression on the plasma membrane in morphine-tolerant rats [28]. The tricyclic antidepressants imipramine was demonstrated to inhibit PKC activity in rat cerebral cortical slices [66]. Furthermore, repeated treatment with desipramine or fluoxetine decreased PKC activity in both soluble and particulate fractions of rat cerebral cortex and hippocampus [67]. We found that amitriptyline inhibited the expression of PKC α , β II, and γ protein in morphine-tolerant rat spinal cord but had no effect on PKA expression [28]. Similarly, Budziszewska *et al.* failed to observe the inhibitory effect of imipramine on PKA expression when they examined glucocorticoid receptor-mediated gene transcription [68]. Moreover, acute amitriptyline treatment inhibited the phospho-PKA and PKC expression in morphine-tolerant rats [28]. These findings suggest that acute amitriptyline treatment preserves morphine's antinociceptive effect in morphine-tolerant rats by inhibiting the expression of phospho-PKA and PKC and by subsequent induction of GLAST and GLT-1 trafficking to the glial cell surface. The latter action enhances EAA uptake from the synaptic cleft and reduces spinal EAA release.

CYTOKINES AND MORPHINE TOLERANCE: EFFECT OF AMITRIPTYLINE

The activation of spinal glia, a characteristic response observed during central neuroimmune activation and neuroinflammation, may mediate and/or modulate the pathogenesis of persistent pain states [6, 69] and CNS neuronal plasticity via neural-glia interaction [70]. In the spinal cord, immune cells (astrocytes, and microglia) might be activated in response to diverse noxious stimuli and pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6. Pro-inflammatory cytokines act in a paracrine fashion; they potentiate cells far from their release site to release pain-related transmitters or substances (e.g. nitric oxide, prostaglandins, and EAAs) from primary afferent terminals [71]. Studies have shown that TNF- α and IL-6 regulate excitatory and inhibitory neurotransmission, respectively, and IL-1 β regulates both excitatory and inhibitory neurotransmission. These cytokines maintain persistent pain by inducing CREB phosphorylation and regulating CREB-mediated gene transcription [72, 73]. TNF- α also shows to stimulate the endogenous intracellular prostaglandins production, thus sensitizing/activating sensory neurons by suppressing the sustained potassium current in nociceptive

DRG neurons [74]. The role of pro-inflammatory cytokines in the development of morphine tolerance is well documented [75, 76]. Chronic morphine administration activates glial cells and up-regulates pro-inflammatory cytokine expression in the spinal cord [77, 78]. Administration of glial metabolic inhibitors fluorocitrate (a nonselective metabolic inhibitor of astrocytes) or propentofylline (xanthine derivative) reverses the development of morphine tolerance in rats [77, 78]. In addition, co-administration of anti-IL-6 antibody, soluble TNF receptors, anti-inflammatory cytokine IL-10, or IL-1 receptor antagonist IL-1ra attenuates opioid tolerance and prevents hyperalgesia and allodynia [6, 78]. Chronic subcutaneous morphine administration increases microglial and astrocytic immunoreactivity and pro-inflammatory cytokine mRNA and protein expression in spinal cord to a greater degree in nerve-injured rats given morphine than in untreated nerve-injured rats [6, 78], indicating a possible synergistic effect of opioid administration on the nerve injury-induced activation of spinal glia cells and cytokine production.

We found that amitriptyline-induced attenuation of morphine tolerance and preservation of morphine's antinociceptive effect might be associated with regulation of spinal pro-inflammatory immune response [29]. Co-administration of amitriptyline with morphine increases the expression of IL-10 in non-activated microglia, activates the p38 mitogen-activated protein kinase (MAPK) pathway (but not ERK, p-ERK, JNK, or p-JUK), and heme oxygenase-1 (HO-1) in neurons, and inhibits the pro-inflammatory cytokine expression in morphine-infused rats [79]. Neutralization of IL-10 expression and blockade of p38 MAPK activation reverses the amitriptyline-induced increase in HO-1 expression and the inhibition of pro-inflammatory cytokine expression. Moreover, inhibition of IL-10 expression, p38 MAPK activation, or HO-1 expression partially blocks the inhibitory effect of amitriptyline on morphine's antinociceptive tolerance. These results suggest that the suppressive effect of amitriptyline on the pro-inflammatory cytokine production in morphine-infused rats is mediated through an increase in IL-10 protein expression in microglia. The IL-10 subsequently activates the neuronal p38 MAPK pathway and increases neuronal HO-1 expression [79].

Studies have suggested that IL-10 and HO-1 produce a positive feedback to amplify the anti-inflammatory effect by up-regulation of carbon monoxide- and p38 MAPK-dependent mechanisms [80, 81]. The transcriptional activation of HO-1 is mediated by the ERK, JNK, and p38 MAPK pathways [82, 83], and p38 MAPK is a key mediator of cellular stresses, such as inflammation and apoptosis. Both *in vitro* and *in vivo* studies have shown that p38 MAPK regulates the production of proinflammatory cytokines, nitric oxide, and prostaglandin E2, by increasing of cytokines release or mRNA transcription [84-86]. As known, increasing of proinflammatory cytokines expression in the spinal cord is associated with neuropathic pain and morphine tolerance [87, 88]. p38 MAPK is activated in neuropathic pain by peripheral inflammation and nerve injury [89-91]. Morphine tolerance associated microglia activation is also activated via p38 MAPK signaling pathway [92]. Inhibition of p38 MAPK was considered to be a potential therapeutic

target for anti-inflammatory therapy [93]. However, contradictory results were reported. Treatment with a p38 MAPK inhibitor resulted in an enhancement of TNF- α production in mast cells [94]. Moreover, inhibition of p38 MAPK resulted in a reduction of cytokine production from mast cells, but increase cytokine release from lipopolysaccharide-activated macrophages [95]. These results suggest that p38 MAPK possesses both pro-inflammatory and anti-inflammatory characteristics, it plays different roles in different inflammatory conditions, depending on the cell type and applied stimulus.

Communication between neurons and glia involves ion flux, neurotransmitters, cell adhesion molecules, and specialized signaling molecules released from synaptic and nonsynaptic regions of neurons [70, 73, 96]. Morphine-induced glia activation and pro-inflammatory cytokine release are also caused by induction of neuronal fractalkine release [87]. Fractalkines, expressed in spinal neurons, produce a diffusible signal that activates nearby microglia, and then release pro-inflammatory cytokines [97]. These results suggest that morphine indirectly affects glia function via chemokines to conduct neuron-glia communication. Activated neuronal PKC γ acts as an important mediator to modulate the neuron-glia communication, which increases astrocyte reactivity following repeated morphine treatment, and this neuron-glia communication may be responsible for the development of morphine tolerance [98]. Together from

our previous studies [79], we suggest that amitriptyline modulates neuroimmune responses via a two-way communication between glial and neurons. Amitriptyline stimulates microglia releasing of anti-inflammatory cytokine IL-10 via a paracrine-manner, and increases neuron *phospho*-p38 MAPK expression, which induces up-regulation of HO-1, via carbon monoxide, and exerts negative feedback control of microglia activity and inhibits pro-inflammatory cytokine expression.

CONCLUSIONS

Currently available medications for the management of chronic pain, in particular for the neuropathic pain, are either inadequate or with unbearable side effects. Tricyclic antidepressants are known to be effective adjuvant for the treatment of chronic neuropathic pain in clinical practice [99, 100]. The data from our laboratory and those of others have shown that the combined treatment of tricyclic antidepressants amitriptyline with morphine preserves the antinociceptive effect of morphine and reduces morphine tolerance. The potential mechanisms by which amitriptyline attenuates morphine tolerance may be involved in inhibition of pro-inflammatory cytokine expression through activation of p38 MAPK pathway and consequently increases neuron HO-1 expression, activation of NF- κ B and prevention of glutamate transporter down-regulation, and even up-regulation of glial GTs GLAST and GLT-1 expression, thus attenuating

Fig. (1). Schematic diagram of proposed cellular mechanisms by which tricyclic antidepressants such as amitriptyline might attenuate the development of morphine tolerance. These mechanisms include: (1) inhibiting the expression of pro-inflammatory cytokines TNF α , IL-1 β , and IL-6 and increasing IL-10 expression via the p38 MAPK-HO-1 signal transduction cascade; (2) activating NF- κ B, preventing glutamate transporter down-regulation, and up-regulating expression of glutamate transporters GLAST and GLT-1 in glial cells; and (3) preventing phospho-PKA and PKC expression, thereby promoting GLAST and GLT-1 trafficking to the glial cell surface. Normal or over-expression of GLAST and GLT-1 in glial cell membranes maintains glutamate transporter uptake activity, reduces spinal excitatory amino acid release, and decreases NMDA receptor activation in spinal cord in morphine-infused rats. Abbreviations: G, G-protein-coupled receptor; M, morphine; glu, glutamate; T, glutamate transporter; NMDA-R, N-methyl-D-aspartate receptor.

the morphine-evoked EAA accumulation in morphine tolerant rat CSF (Fig. 1). Evidence suggests that tricyclic antidepressants such as amitriptyline may be useful as an adjuvant in combination with opioids for the treatment of patients who need long-term opioid treatment for chronic pain management. Preclinical studies have successfully elucidated some of the molecular mechanisms of tricyclic antidepressants and its effects on pain mechanism [101]. Translational research is, as in other domains of medicine, a key for further improvement on the treatment of morphine tolerance with amitriptyline.

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