Molecular mechanisms of opioid tolerance: From opioid receptors to inflammatory mediators (Review)

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Received September 26, 2020; Accepted April 19, 2021

DOI: 10.3892/etm.2021.10437

Abstract. Opioids are considered the most effective analgesics for the treatment of both acute and chronic pain. However, prolonged opioid use can induce a certain level of tolerance to its analgesic effects, leading to a reduction in its effectiveness, addiction and abuse. A better understanding of the mechanisms underlying opioid tolerance may provide insights into this phenomenon and aid in the development of novel methods to combat the side effects of opioid tolerance. The present review focused on two major contributors to tolerance, opioid receptors and inflammatory mediators. The molecular mechanisms involved in the desensitization of the opioid receptors were briefly described, including their phosphorylation, internalisation and recycling. Subsequently, the effects of Toll like receptor 4/NOD-like receptor family pyrin domain containing 3-mediated proinflammatory responses in opioid tolerance were discussed, aiming in supporting the identification of novel therapeutic targets.

Contents

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Key words: pain, opioid tolerance, morphine, opioid receptors, proinflammatory cytokines, Toll like receptor 4, NOD-like receptor family pyrin domain containing 3, neuroinflammation

1. Introduction

Pain is an important global health problem. Recent data suggest that >30% of the population in China suffers from a form of chronic pain, including joint pain, headaches, severe back pain and cancer-related pain, with a low rate of treatment (1). The aetiology of pain is a complex, transdisciplinary process with multiple causes, including cancer, rheumatoid arthritis, spinal problems, injuries and surgery (2). Individuals who experience chronic pain usually have a reduced quality of life resulting from both the physical and mental toll of this condition. For instance, numerous individuals who suffer from chronic pain experience depression, anxiety and even suicidal thoughts (3). Opiates, which were discovered thousands of years ago, are to date the most common and effective analgesics for the treatment of pain and pain-associated disorders (4). Important progress has been made in the development of opioids derived from opiates in the last century, but several of their side effects persist (5). An Italian study revealed that the average duration of opioid treatment in patients with cancer is ~105 days (6). Long-term opioid treatment results in decreased analgesic efficacy via increased tolerance and an increased potential for addiction (7).

Tolerance is defined as the reduction in the effects of a drug following its prolonged administration, leading to the loss of drug potency and the increase in dosage to maintain its analgesic effects (8). However, this increase in dosage may accelerate tolerance and its side effects, including respiratory depression, gastrointestinal immobility and addiction (8,9). The drug interactions with opioid receptors and the dose and frequency of administration are the primary reasons for the development and extension of tolerance (10). Numerous mechanisms are involved in opioid tolerance, including the receptor downregulation and signalling desensitisation, upregulation of drug metabolism and initiation of

compensatory/opponent processes (11). Because of the loss of the analgesic effect and the severity of the side effects, drug tolerance is one of the most challenging issues in the clinical application of opioids and can ultimately lead to poor patient compliance and treatment discontinuation (12). Thus, a better understanding of the molecular mechanisms underlying the development of opioid receptor tolerance, regulation and signal transduction may help in the identification of novel strategies to tackle these clinical problems.

Although it has been widely established that opioid drugs are critical to proper pain management and that their receptor desensitisation is closely associated with opioid tolerance, the role of inflammation and the associated molecular mechanisms in this phenomenon have not been well studied until relatively recently (13). A growing body of literature suggests that activated microglia and astrocytes (glia) are the primary targets in pain management, because of their function in pain transmission and opioid analgesia (14,15). Morphine, one of the most effective analgesics, affects the glia through Toll like receptor 4 (TLR4), inducing proinflammatory cytokine production and increasing morphine tolerance (16). Due to its strong anti-analgesic effects and critical role in morphine tolerance, IL-1β stands out among the morphine-induced cytokines (17). Morphine treatment upregulates IL-1β production, and antagonisation of the IL-1 receptor reverses morphine tolerance (14). Thus, both the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, a major signalling pathway involved in IL-1β release, and TLR4 signalling are receiving increasing attention for the regulation of morphine tolerance (18).

The present review summarized the current knowledge on the interactions between opioid systems and the function of inflammatory factors and their impact on the development of opioid tolerance. The cellular and molecular mechanisms involved in opioid tolerance and the inflammatory factors implicated in TLR4- and NLRP3-inflammasome-mediated tolerance responses were discussed. The aim of the current review was to reveal the full image of opioid receptors and inflammatory factors and their impact on opioid tolerance and aid in the identification of potential therapeutic targets for the enhancement of analgesic efficacy and the reduction of side effects in chronic pain management.

2. Opioid receptors

Opioid receptors are a class of seven-transmembrane-spanning inhibitory G protein-coupled receptors, with a high affinity for β-endorphin and enkephalins and a low affinity for dynorphins (19). They are widely expressed in the pain-modulating descending pathways of various tissues, including the brain, spinal cord, peripheral neurons and digestive tract (20). The activation of these receptors is critical for the analgesic effects of these drugs and is achieved via the direct inhibition of the spinal cord neurons, preventing pain signalling in the spinal cord (7,21). There are four different types of opioid receptors related to analgesia: μ opioid receptors (MORs), δ opioid receptors (DORs), κ opioid receptors (KORs) and opioid receptor like-1 (ORL-1), which are all well characterised at both the molecular and pharmacological levels (22) (Table I).

MORs. MORs are the most common and well-studied opioid receptors in pain management. MORs were the first opioid receptors identified in 1973 (23). In 2012, the first X-ray crystal structure of the murine MOR was characterised. It revealed significant detail about ligand and receptor binding patterns, providing valuable insights for the identification of novel bioactive molecules and the development of better drugs for pain management (24). The phosphorylation of MORs is one of the most significant methods of receptor internalisation and desensitisation, eventually causing opioid tolerance (25). There are >15 serine, threonine and tyrosine residues in MORs, which are accessible for phosphorylation by various protein kinases (26). Ser375 at the C-terminus of the rat MOR is phosphorylated to varying degrees upon treatment with both morphine and [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO; a strong synthetic agonist of MORs) (27). Another study has indicated that the phosphorylation of Tyr166 inhibited the G protein activation mediated by DAMGO (28). In addition, multi-phosphorylation in the specific region of the C-terminal tail of MORs has also been demonstrated to be involved in the endocytosis of MORs (29). Both point mutations and alanine substitution of the serine and threonine residues from 375 to 379 (STANT sequence) has been indicated to markedly decrease the agonist-induced recruitment of β-arrestin and receptor internalisation. However, this did not eliminate the induction of acute desensitisation (29-31), suggesting that multiple amino acid residues are involved in the regulation of MORs. These ligand-mediated differences also indicated that receptor regulation and trafficking vary depending on the agonist and require further elucidation.

DORs. Numerous studies have revealed that DORs and MORs directly interact with each other to form heteromers, which have been confirmed via co-immunoprecipitation (32,33). Interestingly, DOR agonists not only activate DORs, but also induce MOR internalisation and degradation, thereby inhibiting MOR agonist activity and inducing morphine tolerance (34). It has been suggested that blocking DOR function via gene knockout or DOR antagonists may reduce morphine tolerance (33,35-38). However, DOR knockout in mice presented conflicting results with either no alteration (39) or in certain cases, reduced morphine analgesic effects (40). This may be due to the high amino acid sequence similarity (~60%) in murine DOR and MOR proteins. High doses of their specific ligands can bind to both receptors and enhance the analgesic effect (35). Similar to MORs, phosphorylation of the C-terminal residues results in DOR desensitisation and regulation. G protein-coupled receptor kinase (GRK) 2 phosphorylates the Ser363 residue, which is a key event in DOR regulation (41). Thr353, at the COOH-terminal tail, is critical for the downregulation of DORs in response to [D-Ala^2, D-Leu^5]-enkephalin (42). The phosphorylation of Thr161 by cyclin-dependent kinase 5 is required for DOR expression and the production of MOR-DOR heterodimers (43), and serves an important role in the development of morphine tolerance. Taken together, these studies suggested that DOR phosphorylation may attenuate opioid tolerance during pain management.

KORs. KORs represent another appealing therapeutic target activated by endogenous dynorphin, a specific endogenous

Table I. Opioid receptor type classification.

Opioid receptor class	Location	Endogenous ligands	Clinical effects	Agonists	Antagonists
μ opioid receptor	Brain, spinal cord, mesenteric plexus, sub-mucosal plexus	β-endorphin, enkephalins, endomorphins	Analgesia, sedation, mood alteration, constipation, nausea	DAMGO, morphine, fentanyl, endomorphins, beta-endorphin	Naloxone
δ opioid receptor	Mesenteric plexus, brain	Enkephalins, β-endorphin	Analgesia, respiratory depression, antidepression, seizures	DPDPE, SNC-80, deltorphin, β-endorphin	Naltrindole
κ opioid receptor	Brain, spinal cord, mesenteric plexus	Dynorphin A, dynophin B	Analgesia, dysphoria, hallucinations	Bremazocine, dynorphin	Naloxone, norbinaltorphimine
Opioid receptor like-1	Brain, spinal cord, smooth muscles, peripheral ganglia, immune system	Nociceptin/orphanin FQ	Analgesia, anti-allodynia	Nociceptin, MCOPPB	Naloxone

DAMGO, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin; DPDPE, [D-Pen^2, D-Pen^5]-enkephalin.

ligand of KOR. KORs are phosphorylated, desensitised and internalised by their agonists (44). Salvinorin A, nalfurafine hydrochloride and type II thioesterase from the rifamycin biosynthetic pathway, which are three structurally distinct kappa ligands, induce KOR internalisation in a dose-dependent manner with different potency rankings (45). The phosphorylation of Ser369 mediated by GRK and β-arrestin binding causes KOR desensitisation and sustains analgesic tolerance. These results were confirmed both *in vitro* in transfected cells and *in vivo* using mice (46). In addition to its analgesic benefit, KOR signalling also plays several other important roles across the nervous system. It is involved in the mediation of negative emotional states, such as drug reinstatement, depression and aversion (47-50), and its natural agonist, salvinorin A, functions as a psychoactive drug (51,52).

ORL-1. ORL-1 is also known as the nociception opioid peptide receptor. It is the most recently discovered opioid receptor and is known to bind to its natural ligand nociceptin, a 17-amino acid neuropeptide (53). Although ORL-1 shares high sequence identity with the classical opioid receptors (μ, δ and κ), ORL-1 ligands possess low affinity for these other opioid receptors (54,55). Reciprocally, agonists of the classic opioid receptors, such as opioid peptides or morphine-like compounds, possess low or no affinity for ORL-1 (56). Both nociceptin and the ORL-1 agonist Ro646198 induce rapid internalisation of ORL-1 in a concentration-dependent manner within minutes of exposure, much faster than that of the other three receptors (57), although they share a similar internalisation mechanism. ORL-1 plays a critical role in the regulation of several brain activities, including instinctive and emotional behaviours (53). Studies on ORL-1 have indicated its potential therapeutic use for non-addictive painkillers, depression and Parkinson's disease in the future (58).

3. Molecular mechanisms of opioid tolerance

The molecular mechanisms underlying *in vivo* opioid tolerance remain uncertain; however, numerous studies have proposed that the regulation of opioid receptors via mechanisms, such as desensitisation, phosphorylation, β-arrestin binding, endocytosis, re-sensitisation and recycling, is involved in the development of opioid tolerance (Fig. 1). For example, when the receptors are activated by the endogenous μ-opioid peptide endorphin or exogenous opioid agonists, such as morphine, the Gα and Gβγ subunits dissociate from each other and bind to potassium and calcium channels. This mechanism modulates voltage activation, inwardly rectifies the potassium channels and inhibits calcium conductance, inducing cellular hyperpolarisation and inhibiting tonic neural activity (59-61). A previous study using *in vitro* and *in vivo* model systems have demonstrated that the primary action of opioid receptors is to positively regulate potassium channels and negatively modulate calcium channels in the nervous system, from neurons in the hippocampus to the dorsal root ganglia (62). Subsequently, MORs are phosphorylated by GRK at different amino acid residues, with a saturation time of <20 sec (25). In addition, β-arrestin serves a critical role in the development of opioid tolerance. In mice, lack of β-arrestin-2 enhanced morphine analgesic function and attenuated the development of morphine

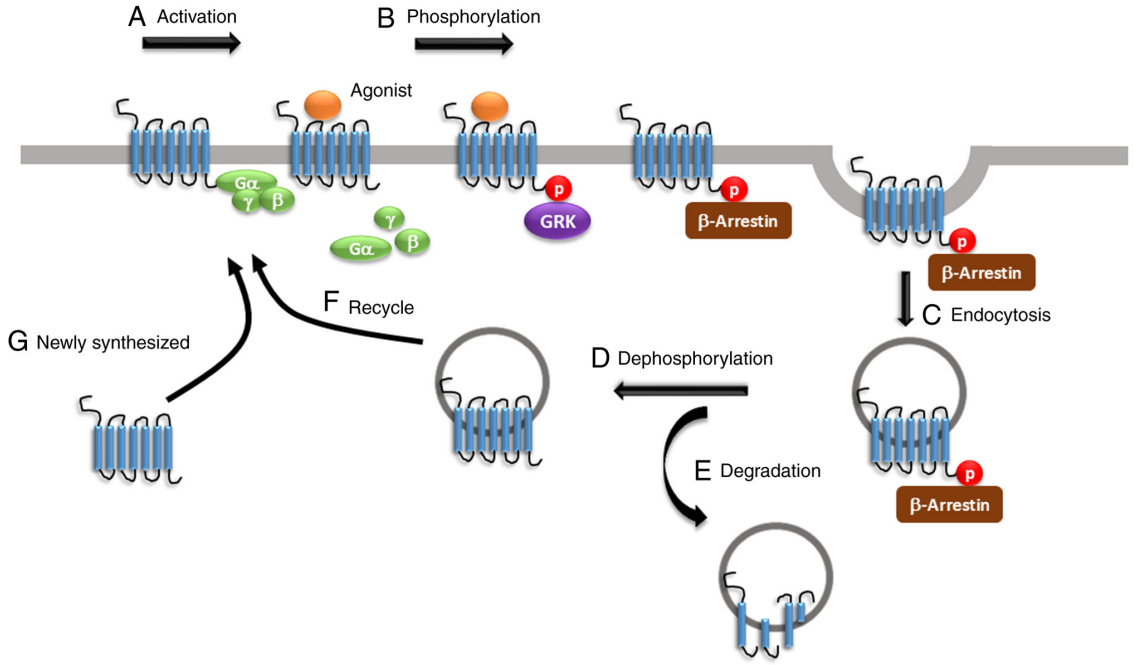

Figure 1. Regulation of MORs. (A) The activation of MORs is dependent on the binding of agonist to receptors. (B) After binding, Gα and Gβγ subunits dissociate from each other. GRKs phosphorylate receptors. After phosphorylation, β-arrestin is recruited to the ligand-receptor complex to prevent further G-protein coupling. (C) The receptors together with β-arrestin then undergo endocytosis into the early endosomes, which is called receptor internalization. Receptors can be (D) dephosphorylated and (E) degraded by lysosomes or (F) resensitized through trafficking back to the cell membrane. (G) The newly synthesized receptors are transferred to the membrane for further ligand-receptor activation. MOR, μ opioid receptor; GRK, G protein-coupled receptor kinase; p, phosphorylated.

tolerance (63). The phosphorylation of the GRK family and β-arrestin binding triggers clathrin-dynamin-mediated MOR endocytosis (25). In addition, arrestin-dependent internalisation is considered the first step in receptor recovery following desensitisation, resulting in the translocation of reactivated receptors to the plasma membrane through recycling. This event is known as resensitisation (25). A reduction in opioid receptor numbers, resulting from a decrease in receptor synthesis or receptor internalisation followed by degradation, also contributes to opioid tolerance (25). *In vivo* studies indicated that chronic treatment with opioids promoted the downregulation and upregulation of opioid receptors (64-66), with tolerance usually associated with receptor downregulation.

4. Proinflammatory cytokines in morphine tolerance

The two possible mechanisms involved in drug tolerance are within-system and between-system adaptation (67). Within-system drug tolerance occurs when opposite reactions are elicited within the same system. A recent study has indicated that chronic morphine use induced a shift in MOR signalling from the predominantly inhibitory Gi/Go adenylyl cyclase to the stimulatory Gs adenylyl cyclase through the upregulation of different MOR receptor variants (68). Specifically, chronic morphine use induced the phosphorylation of the carboxyl terminal sites on MOR-1B2 and MOR-1C1, enhancing Gs protein association (68). In between-system adaptation, different drug-sensitive systems are linked to the drugs' primary action system. Neuroinflammation and the release of proinflammatory cytokines, as well as innate immune signalling, such as TLR4- and NLRP3-mediated inflammasomes, are the key molecular mechanisms for between-system adaptation (Fig. 2) (69).

Glial cells are activated by cytokines in the central nervous system and release other mediators that trigger neuroinflammation (70). IL-1β, a key proinflammatory cytokine, plays an important role in host defence and inflammation and is a critical mediator of inflammatory pain and opioid analgesia (71). In a mouse study, the administration of IL-1β abolished morphine analgesia, and genetic or pharmacological inhibition of IL-1 signalling prevented the development of morphine tolerance (72). Prolonged morphine treatment was indicated to induce glial TLR4 activation, which promoted neurotoxicity and amplified nociceptive signalling in the spinal cord (73). Moreover, chronic morphine use activated TLR4 signalling in the brain, especially in the periaqueductal grey region, resulting in changes in inflammatory cytokine expression, thereby inducing glutamatergic signalling and eventually leading to opioid tolerance (74). Expression of IL-1β could also be induced by morphine via activated microglia, which disrupted glutamate homeostasis by downregulating glutamate transporter 1 and increasing glutamate, thereby triggering the release of ATP from the glia. These events may contribute to excitotoxicity and chronic inflammation, which leads to continued morphine discontinuation (75). Interestingly, IL-1β treatment significantly upregulated MOR mRNA expression in various cell types, including primary astrocytes, neurons and microvascular endothelial cells, which further supports the interaction between IL-1β and the opioid system (76,77). In addition, other inflammatory cytokines, such as TNF-α, IFN-α, IL-4 and IL-6 are also associated with MOR expression in neural and immune cells (78). IL-1β mediates its function through IL-1

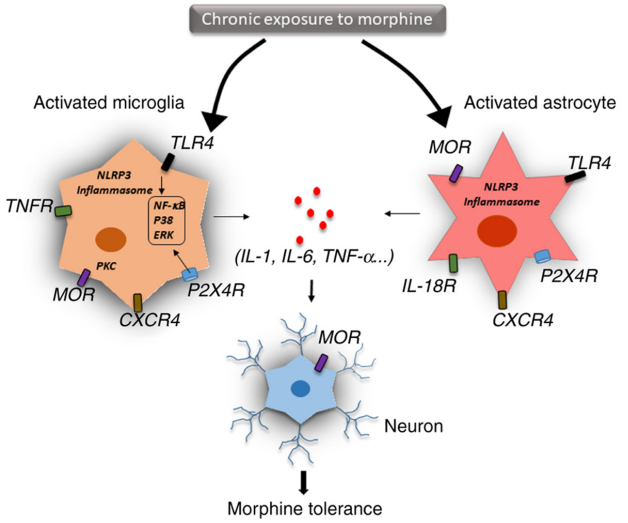

Figure 2. Central immune mechanisms of opioid tolerance. Neuroinflammation and the release of proinflammatory cytokines are the key factors of the central immune mechanisms of opioid tolerance. After chronic exposure to morphine, microglia and astrocytes are activated via different immune signalling pathways. Activation of TLR4/NF-κB signalling, the NLRP3-dependent inflammasome and the purinergic receptor P2X4R have been demonstrated to induce the production of proinflammatory cytokines, such as TNFα, IL-18, IL-1β and IL-6, in microglia and astrocytes. These inflammatory cytokines are associated with alteration of the MOR level in neural and other immune cells. Cytokine production is mainly mediated by NF-κB activation, p38 phosphorylation and ERK signalling. IL-18 derived from microglia signals through IL-18R, resulting in D-serine release from astrocytes, which then induces N-methyl-D-aspartate receptor activation in neurons. The chemokine receptor CXCR4 induces desensitization of MORs in neurons, leading to opioid tolerance. TLR4, toll-like receptor 4; NLRP3, NOD-like receptor family pyrin domain containing 3; P2X4R, P2X purinoceptor 4; MOR, μ opioid receptor; CXCR4, C-X-C chemokine receptor type 4; TNFR, TNF receptor; IL-18R, IL-18 receptor.

receptor type 1 protein (78). Previous studies have indicated that IL-1β stimulation activated downstream signalling, including the janus kinase-STAT, MAPK and NF-κB pathways, which altered MOR transcriptional level (79,80). Chronic morphine treatment has been indicated to upregulate purinergic P2X7 receptor (P2X7R) and increase the expression of IL-18 in the microglia, IL-18 receptor in astrocytes and protein kinase Cγ (PKCγ) in neurons of the spinal dorsal horn. Thus, targeting the P2X7R/IL-18/PKCγ cascade may present a novel therapeutic target for reducing and understanding morphine tolerance in the clinical management of chronic pain (81).

Since IL-1β and IL-18 are both classical cytokines released during inflammasome activation, TLR4 and NLRP3 signalling may be critical for opioid tolerance and should be investigated more thoroughly (18). TLR4, a member of the toll-like receptor family, can recognise specific danger-associated molecular patterns and initiate an immune response (82,83). A previous study reported that morphine resulted in microglial activation via TLR4/myeloid differentiation factor 2 binding (84). Blocking TLR4 signalling inhibited microglial activation, attenuated morphine tolerance and facilitated pain management (85). It is well understood that TLR4 functions as a prime signal that triggers downstream signalling pathways, enhancing the transcription of NLRP3 and pro-IL-1β (86). Subsequently, a second signal triggers several NLRP3 subunits into forming a protein complex known as the inflammasome, which then recruits caspase-1, and eventually leads to the maturation and secretion of IL-1β and IL-18 (87). Numerous studies have suggested that the NLRP3 inflammasome plays an important role in pain conditions, such as post-herpetic neuralgia, postoperative pain and neuropathic pain through secreted proinflammatory cytokines (88,89). It has been demonstrated that inhibition of the NLRP3 inflammasome attenuated morphine tolerance (90). A previous study reported that morphine activated the potassium ATP channel, and blocking this channel alleviated morphine tolerance by inhibiting the heat shock protein 70/TLR4/NLRP3 cascade-mediated neuroinflammation (91). Taken together, these results suggested that TLR4/NLRP3 inflammasome-mediated neuroinflammation is critical for morphine tolerance and pain management.

5. Conclusions

The present review summarized the underlying molecular mechanisms of opioid tolerance facilitated by both within-system and between-system regulation. Agonist activation results in the phosphorylation of the opioid receptors by various kinases, including GRK and PKC, leading to G protein uncoupling, β-arrestin binding, receptor desensitisation and endocytosis, followed by degradation or recycling. Drugs targeting opioid receptors and inhibiting G protein dissociation or β-arrestin binding are still in preclinical or early clinical studies designed to evaluate their impact on opioid tolerance (25). Interestingly, prolonged opioid treatment accelerates the production and secretion of proinflammatory cytokines, such as IL-1β and IL-18, which suppress morphine analgesia and lead to opioid tolerance (14). Inflammatory immune responses are considered a critical contributor to the regulation of the opioid receptors. Numerous studies have suggested that suppression of the TLR4/NLRP3-mediated inflammasome activation and the inflammatory response in microglia lead to an important attenuation of morphine tolerance (18). Taken together, these results could reveal novel therapeutic targets of anti-opioid tolerance, from opioid receptors to inflammatory factors. Several studies have indicated that inflammatory factors promote morphine tolerance; however, how inflammatory factors act on opioid receptors and how they affect opioid analgetic tolerance is still unknown (8,78,92). A better understanding of the association between inflammatory factors and opioid receptors will help understand the mechanism underlying opioid resistance and assist in the identification of potential therapeutic targets, the development of anti-opioid drugs and the treatment of its side effects. There is abundant basic information describing the mechanism and treatment of morphine tolerance, but there are few clinical trials completed to draw definitive conclusions (93), indicating that there is still a lack of effective measures and methods to inhibit morphine tolerance. Multicentre randomised controlled clinical trials are needed in the future. With the development of biomarkers and genetic diagnostic tests, opioid treatment could be personalized, which may aid in addressing opioid tolerance and improving therapeutic outcomes for individual patients. With a deepened understanding of the opioid lifecycle and the underlying molecular mechanisms of morphine tolerance, more effective drugs with fewer side effects may be produced in the future.

Acknowledgements

Not applicable.

Funding

The present study was supported by Natural Science Foundation of Zhejiang Province (grant nos. LQ21H270008 and LQ18H270001), Zhejiang Province Top Discipline of Chinese Medicine (grant no. ZTK2017A02) and Innovation Fund for Youth of Zhejiang Chinese Medical University (grant no. KC201947).

Availability of data and materials

Not applicable.

Authors' contributions

JZ led the present review. YJ, YL and JiF contributed to the conception of the review. JZ and YL developed the research methods, according to the comments and feedback from YL and RM. JZ, JuF and XS performed the literature search and applied the selection criteria. JZ and RM synthesised the data and wrote the first draft of the manuscript. RM, JD and JiF contributed to the modification of the first draft of the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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