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Molecular mechanisms of morphine tolerance and dependence; novel insights and future perspectives

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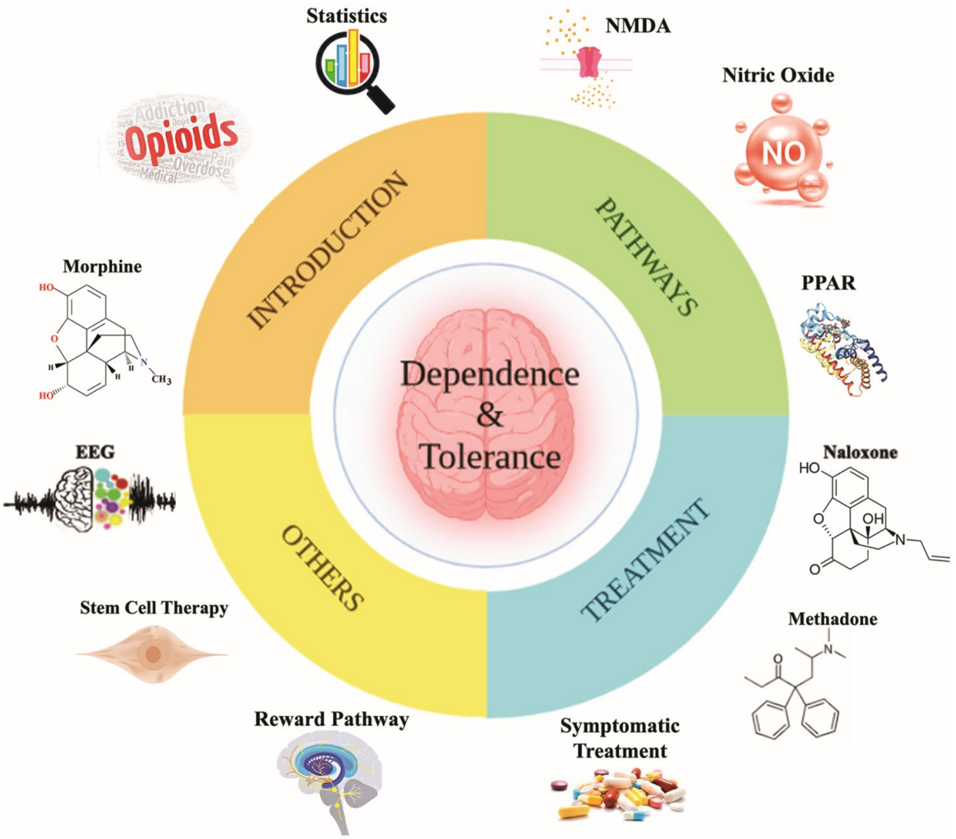
Abstract

Drug addiction is a devastating condition that poses a serious burden on the society. The use of some drugs like morphine for their tremendous analgesic properties is also accompanied with developing tolerance, dependence and the withdrawal symptoms. These symptoms are frequently severe enough to reinforce the person in recovery to start over the use of drug again and hinder the clinical use of drugs like morphine for chronic pain. Research into opioid receptors and related molecular pathways has seen resurgence in the wake of the growing opioid epidemic. The current study provides a comprehensive scientific exploration of the molecular mechanisms and underlying signalling in morphine tolerance and dependence. It also critically evaluates current therapeutic approaches, shedding light on their efficacy and limitations, and future prospects.

Extended author information available on the last page of the article

Graphical Abstract

The graphical abstract depicts an overview of the pathways involved in the emergence of morphine-related tolerance and dependence including NMDA, Nitric oxide, and PPAR, as well as behavioural sensitization along with present and future innovative treatment strategies including stem cell therapy that have been discussed in the current manuscript.



Keywords Addiction · Opioids · Morphine · Tolerance · Dependence · NMDA · Receptor desensitization

Introduction

Morphine was first isolated by a German pharmacist Friedrich Sertürner and initially named morphium after "Morpheus" in Greek mythology meaning God of dreams. Due to its insomniac potential and accidental relief of terrible toothache, Sertürner deemed morphine safe for human consumption. Based on his experiments, he even recommended a safe dose of the morphine for human use [1]. Isolation of morphine from opium poppy not only reduced the dangers associated with the use of raw poppy for pain alleviation but was also the first alkaloid isolated from plant extracts.

Drug addiction is a chronic recurring illness marked by obsessive drug-seeking behaviour, tolerance, reliance, and heightened sensitivity to the drug effects [2]. Addiction usually results in the increased consumption of drug despite knowing debilitating consequences [3]. In US, over 16 million people suffer from addictive disorders, of which 1 to 2 million are addicted to heroin and other opioids [4]. A substantial increase in opioid overdose-related deaths has been observed including fentanyl, tramadol, and prescription drugs like oxycodone [5, 6]. Morphine is widely used for the alleviation of pain in various ailments such as cancer due to rapid onset and prompt relief. However, the chronic use of morphine might lead to the development of tolerance and dependence with detrimental health effects. Physical dependence is characterized by neuronal adaptations that occur due to persistent drug exposure, resulting in symptoms termed as withdrawal effects upon cessation or administration of antagonists such as naloxone [7, 8]. These symptoms might include anorexia, cough, abdominal pain, diarrhoea, sneezing, runny nose, anxiety and even death if exacerbated [9, 10]. Under normal circumstances, these symptoms are associated with unrest that significantly alters the quality of life and may have other adverse effects such as memory impairment. In animal models of morphine dependence, withdrawal symptoms appear in the form of jumping, diarrhoea, rearing, teeth chattering, forepaw tremors and wet dog shakes. These models are valuable tools for observing the extent of morphine dependence and withdrawal [11, 12].

Another associated effect with the prolonged use of morphine apart from physical dependence is drug tolerance. Tolerance is a physiological state that is characterized by decreased response to the drug after repeated exposure, often resulting in dose escalation to get the same relief or effect as with the previous minimal dose [13]. Hence, the use of morphine as an analgesic is hindered by adaptive changes such as addiction, dependence, tolerance and associated side effects [14]. Most of the intended pharmacological activities of morphine such as analgesic, euphoric, and sedative effects are compromised, while the unintended effects such as respiratory depression and nausea are potentiated when the dose of morphine is increased. Tolerance might be of three different types including, pharmacokinetic, pharmacodynamic and learned tolerance. Any changes in distribution and metabolism of opioids are referred to as pharmacokinetic tolerance that might be of genetic origin or after prolonged use. The pharmacodynamic tolerance is usually associated with the mechanistic neuroadaptive changes at cellular level. It also includes the transient changes where receptor response and density may profoundly decline either due to desensitization or internalization. Tolerance may also develop due to overexpression of intracellular second messengers such as nitric oxide synthase (NOS), cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP), thus causing alteration in free intracellular calcium concentration [15–17]. Learned tolerance refers to a decline in the drug effect, which is due to the compensatory mechanisms that are learned [16]. In animal model of tolerance, behavioural studies such as tail immersion test [18], tail-flick test and hot-plate test [19] are frequently employed to investigate the effect of test molecules.

The development of tolerance and dependence after repeated use of morphine for clinical or recreational purposes might lead to the addictive or pseudo-addictive behaviour in individuals undergoing discontinuation/detoxification of the drug. Hence, it remains crucial to understand the mechanisms underlying the development of such conditions in order to identify newer drug targets and develop future drug candidates. Therefore, the current report elucidates the molecular intricacies associated with the development and expression of morphine tolerance and dependence.

Overview of opioid receptors

Morphine is an alkaloid and acts on opioid 7-transmembrane G protein coupled receptors having extracellular amino and intracellular carboxylic domains. G protein receptors are distributed throughout the body within the central as well as peripheral nervous systems. Peripherally, opioid receptors exist in gastrointestinal and respiratory tract where they regulate motility and secretions [20, 21]. These receptors are categorized into three main types namely; mu opioid receptors (MOR), kappa opioid receptors (KOR), delta opioid receptors (DOR) and other opioid-related receptors such as Nociceptin/Orphanin FQ (NOP) receptors (NOR) located in central nervous system, gastrointestinal system and sensory nerves. Among them, MORs are primarily associated with analgesic effects, euphoria, dependence, respiratory depression, constipation, miosis, and vasodilation. Agonism of the MOR results in the inhibition of gastric emptying, increasing pyloric muscle tone and delaying passage through intestine ultimately leading to constipation. In the respiratory system, morphine acts on respiratory centre within the brainstem [22]. This antitussive effect of opioids such as morphine is primarily mediated through the MOR and KOR. [23]. KORs are also located in central and peripheral nervous system and are associated with analgesia, diuresis, and dysphoria. DORs are similarly expressed in the brain and spinal cord and are associated with analgesia. NORs are an intriguing class of opioid-related receptors that play important roles ranging from analgesia to hyperalgesia. Interestingly, despite sharing structural and functional commonalities with opioid receptors, NOP receptors are distinct in that they bind the endogenous peptide nociceptin/orphanin. [22, 24]. ZORs are widely distributed in the body organs including heart, liver, brain, pancreas, muscles and kidney and are known to contribute in the development and regulation of normal and tumour cells [25]. Notably, MOR agonists, including morphine analogues and other structurally diverse compounds, comprise the majority of clinically relevant drugs targeting opioid receptors.

It is important to acknowledge that despite the status of morphine as “goal standard” analgesic and a prototypical MOR probe, MOR agonists exhibit quite diverse receptor pharmacodynamics. Indeed, the landscape of opioid signalling is quite intricate in terms of diverse array of responses of different agonists which are further complicated by ligand-dependant signalling phenomena such as internalization and modulation by arrestins 1 and 2. Apart from morphine, ligand-dependent signalling of MOR agonists is a highly fascinating area of research. For instance, fentanyl, a synthetic opioid agonist, has a high affinity for

MOR and exhibits its analgesic effect through the activation of G protein signalling, but with a distinct bias when compared to morphine. Fentanyl has been shown to recruit β-arrestin-2 less efficiently than morphine, which might contribute to its increased analgesic potency and reduced side effect like constipation and dependence [26]. Endogenous opioids such as endomorphins (endomorphin-1 and endomorphin-2) also exhibit interesting signalling properties. They possess high selectivity and affinity for MOR and stimulate both G protein and β-arrestin pathways, leading to potent analgesic effects [27]. Additionally, the endogenous peptide β-endorphin has been shown to promote analgesia through MOR-mediated G protein signaling, with a reduced propensity to recruit β-arrestin-2, similar to fentanyl [27]. More recently, novel MOR agonists with "biased agonism" or "functional selectivity" have been developed. These drugs, such as oliceridine (TRV130), have been designed to preferentially activate G protein signalling pathways over β-arrestin pathways [28]. This biased agonism is believed to provide potent analgesia while reducing adverse effects typically associated with opioid therapy such as respiratory depression and constipation [28, 29]. In addition to the aforementioned agonists, buprenorphine, a partial MOR agonist, has been extensively studied for its unique signalling properties. The partial agonist nature of this agent at MOR results in a ceiling effect for respiratory depression which significantly improves its safety profile [30]. Buprenorphine has also been suggested to act as a “biased agonist”, preferentially recruiting and activating G protein over β-arrestin pathways [26]. Furthermore, an investigational opioid, PZM21, offers an interesting perspective into the realm of ligand-dependent signaling of MOR agonists. PZM21, discovered via structure-based drug design have demonstrated a unique signaling profile that selectively activates G protein pathway while minimally recruiting β-arrestin-2 which results in potent analgesic effects with less dependence and tolerance [31]. Worth mentioning, several natural products, such as alkaloid mitragynine from the plant *Mitragyna speciosa* have also been demonstrated to have multifaceted interaction with the MOR. Mitragynine is thought to act as a partial MOR agonist, but may also interact with other receptor systems, suggesting potential as a novel multitarget analgesic agent [32]. Overall, the landscape of MOR agonist signalling is diverse and complex and offers opportunities for the development of novel therapeutics with improved efficacy and safety profiles.

Molecular mechanisms involved in morphine tolerance and dependence

Receptor desensitization

Desensitization refers to decreased response either due to internalization of receptors or decreased activity of surface receptors following the repeated use of morphine or its analogues. It has been reported that acute dose of morphine reduces the cyclic adenosine monophosphate (cAMP) and leads to hyperpolarization by altering calcium and potassium ion channels [33]. However, chronic morphine use might bring adaptive changes observed within opioid receptors, thus decreasing receptor response [34]. The trafficking of opioid receptors, particularly MORs, plays a crucial role in the development of morphine tolerance [35]. Changes in receptor transport essentially correlate with the dynamic movement of these receptors to and from the cell surface. Prolonged exposure to morphine has been shown to alter receptor transport, leading to a reduction in the number of available surface receptors and thus contributing to morphine tolerance [36]. Internalization of receptors was thought to be involved in tolerance development but *in-vitro* studies showed that strong development of tolerance with morphine was associated with poor internalization of MORs [37]. Tolerance may also involve desensitization of MORs [37]. Calcium and potassium ion channels take part in rapid desensitization, while enzyme activity is observed in sustained type of desensitization. Increased activity of adenylate cyclase and elevated levels of cAMP affecting cAMP response element binding protein (CREB) have also been reported. MOR desensitization is also linked with the cytosolic protein (β-arrestin-1 and β-arrestin-2) deregulation [38]. These β-arrestin proteins are attached to opioid receptors and alter their phosphorylation via serine/threonine kinases. Cell signalling through second messengers is inhibited by the activation of β-arrestin and ultimately leads to receptor desensitization [39]. Phosphorylation of Mitogen-activated protein kinases (MAPks) has also been implicated in receptor desensitization. Large numbers of potential substrates including transcription factors are under the control of MAPKs gene expression. Extracellular signal-regulated kinases (ERK1/2) are thought to have a strong effect in chronic morphine exposure. However, debate still remains as some researchers have observed increase in ERK1/2 phosphorylation, while others suggested no effect upon chronic morphine exposure [40]. In addition, Gut microbiota has also been reported to influence morphine tolerance. Microbiota has been increasingly recognized for its role in drug metabolism and response. Current evidence suggests that changes in the gut microbiota may also play a role in the development of morphine tolerance [41]. Morphine can change the composition of the gut microbiota, which in turn can affect the metabolism and effectiveness of the drug.

While discussing every mechanism of tolerance known to all opioids is beyond the scope of this review, it is crucial to highlight that not all opioid analgesics follow the same mechanisms as morphine during the development of tolerance and dependence. For instance, Fentanyl is known to cause a strong and rapid internalization of MOR upon administration resulting in distinct downstream signalling and tolerance mechanisms [42]. Key roles of β-arrestin 1 and β-arrestin 2 in opioid receptor desensitization and internalization and subsequent opioid tolerance have been suggested, particularly for opioids with high intrinsic efficacy such as fentanyl and etorphine [43]. Indeed, fentanyl has been shown to recruit β-arrestin-2 less efficiently than morphine, which might contribute to its increased analgesic potency and reduced side effects. This complex interplay with arrestin proteins can lead to divergent signalling cascades and subsequent tolerance profiles [43, 44]. Methadone is a unique full MOR agonist with a long duration of action. Methadone binds strongly to the opioid receptors and has slow receptor dissociation kinetics that result in prolonged receptor activation. This slow onset and offset of effects along with the N-methyl-D-aspartate receptor blocking property might contribute to its ability to prevent withdrawal symptoms and cravings in individuals with opioid dependence [45]. Decreasing plasma concentrations of methodone have been reported during the maintenance phase (resulting in tolerance) possibly occurring via autoinduction of hepatic microsomal enzymes [46]. The case of Tramadol is interesting in that this agent has been shown to act via both weak agonism of MOR and inhibition of the serotonin reuptake [47]. The inhibition of serotonin and norepinephrine reuptake by tramadol has the potential to augment pain relief and potentially decrease the need for relying solely on activation of opioid receptors. This dual mechanism may be responsible for the different pattern of tolerance than agents that act via MOR only like morphine [47].

Role of oxidative stress and nitric oxide pathway

Nitric oxide (NO) is synthesised from L-ARG (L-arginine) by the action of nitric oxide synthase (NOS). NO is a neurotransmitter and plays its part in signalling pathway between neuron, glial cells and cerebral blood vessels. The enzyme NOS has three isoforms: iNOS (inducible nitric oxide synthase), eNOS (endothelial nitric oxide synthase) and nNOS (neuronal nitric oxide synthase) [48]. Morphine can induce free radical formation or it may reduce the antioxidant defence thus causing damage to the cells [49]. It has been shown that morphine results in decline in glutathione (GSH) levels in brains of rodents and humans [49]. Depletion in GSH in the caudate nucleus upon morphine injection has also been reported. Acute decline in GSH level in cerebrospinal fluid was observed in patients experiencing cancer-related pain following morphine administration [50]. Abdel-Zaher et al. have reported that repeated administration of morphine decreased GSH level in the mouse brain [51]. Chronic morphine usage has also been shown to interfere with the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-peroxidase (GSHPx) [49]. The cellular homeostasis involving redox balance can be disrupted by morphine but the extent to which it can cause changes depends on many factors like species, age, sex, type of tissue, dose, duration of use and interaction with other drugs [49].

Peroxynitrite (PN) is an oxygen specie formed from the precursors superoxide and nitric oxide which are involved in pain modulation, opiate-induced analgesic effect and antinociceptive tolerance [14, 49]. NOS, NADPH-oxidase and spinal manganese superoxide dismutase (MnSOD) are also involved in the formation of PN and its precursors [49]. The cellular processes such as activation of neuroimmune response and the release of pro-inflammatory cytokines like TNF-α, interleukin 6, interleukin 1β activation and N-methyl-D-aspartate (NMDA) receptors can also take part in PN formation and thus morphine dependence and tolerance [49]. PN is also required for the activation of NF-κB pathway through indirect inhibition of mitochondrial respiration and by direct redox-sensitive interactions [14]. The activation of NOS and NADPH-oxidase result in NO and superoxide production, respectively, while inactivation of MnSOD results in PN formation. The inhibition of the NOS and MnSOD activation has been shown to prevent the morphine-induced antinociceptive tolerance [49]. NADPH-oxidase inactivation can be inhibited by the morphine and PN scavengers which are the cationic Mn (III) N-alkylpyridylporphyrins [49]. Thioredoxin-1 (Trx-1), which is a redox regulating protein and a neurotrophic cofactor has been shown to inhibit the tolerance to the analgesic effects of morphine [52]. Morphine-induced increase in expression of Trx-1 has been demonstrated [52]. However, the studies on Trx-1 in relation to morphine are very limited especially in the rodent models.

Alterations in NO synthesis have been linked to various neurological diseases. Cerebral injury or reperfusion and degenerative process trigger glutamate release leading to increase in NO via nNOS. NO binds to guanylyl cyclase, the cGMP-producing enzyme, resulting in the activation of protein kinase G (PKG) and synaptophysin phosphorylation which result in the potentiation of Glu-ergic neurotransmission. Effect of NO on inhibitory GABA-ergic synaptic transmission has also been reported [53]. The effects of NOS inhibitors and NO precursor on expression and development of morphine dependence and tolerance were investigated in various research studies. Opioid tolerance and dependence is biphasic pattern having two phases: induction and expression. It has been reported that antinociceptive tolerance was attenuated in NOS-deficient mice [54]. Moreover, the administration of methylene blue (a sGC inhibitor) and L-NAME (a NOS inhibitor) decreased the development of morphine antinociceptive tolerance, observed during behavioural tests like Tail-flick and Hot-plate test [55]. In morphine-dependent mice, both L-NOARG and L-NAME (NO synthase inhibitors) reduced the naloxone-precipitated withdrawal jumping and diarrhoea (Cappendijk et al. 1993). It was proposed that the decreased activity of NO might be responsible for anti-withdrawal effect through excitatory amino acid (EAA) receptors. In a similar association to excitatory neurotransmitters, noradrenaline has also been reported to be involved in the onset of withdrawal symptoms. Nitric oxide tends to increase tyrosine hydroxylase and release of presynaptic catecholamine which might reduce the effect of morphine withdrawal. For this reason the hyperactive noradrenergic system has been perceived in opioid withdrawal syndrome [56]. In another study, it was reported that hyperbaric oxygen (HBO_2) modulated monoaminergic neurotransmission and NO-cGMP pathway and alleviated morphine withdrawal symptoms [57]. Hence, the role and involvement of cGMP in development of morphine-induced antinociceptive tolerance is closely related to NO [58, 59]. Abnormal excitation in neurons or synapses within CNS due to activation of NO-cGMP pathway was found to be associated with chronic pain. Interestingly, L-arginine could mimic the chronic pain by activating NO-cyclic GMP pathway [58, 59]. NO-cGMP signalling is also thought to be involved in reward memory [57]. Therefore, the NOS inhibitors might curtail the expression of morphine dependence by suppressing the NO-cyclic GMP system, which is considered to mediate the effects of excitatory amino acid receptor activation [54].

Role of NMDA receptors

Glutamate receptors are widely distributed in CNS and are involved in rapid excitatory synaptic transmission. NMDA receptors have been reported to be involved in neuronal development, memory formation, learning and long-term potentiation. Either hyperactivity or hypofunction of NMDARs can contribute to disease pathology and these receptors are involved in several nervous system disorders like ischemic brain injury, neurodegenerative diseases, depression, schizophrenia and pain [60]. Interestingly, NMDA antagonist may block learning processes but not the established memories [61]. Chronic morphine administration has been shown to induce dependence and tolerance and elevated NMDA receptor activity in mice brain [62]. Dependence and tolerance is also postulated to be associated with the changes in neuronal plasticity, much like the learning processes. While repeated and prolonged exposure to opioids might result in neuronal adoptions, inhibition of learning process by NMDAR antagonists could be associated with interesting outcomes. Hence, opiate dependence and tolerance can be affected by the administration of NMDA antagonists which may attenuate or block behavioural changes associated with withdrawal of morphine precipitated by naloxone administration. One such agent is MK-801 (dizocilpine), where it was observed that aversion in rats following morphine withdrawal was minimized after co-treatment with this antagonist [63]. During this study, morphine exhibited analgesic effect in both saline and MK-801 treated animals. However, saline-treated group developed rapid tolerance as compared to the MK-801 after prolonged use of morphine [63]. Interestingly, animals co-treated with MK-801 exhibited less withdrawal effects as compared to saline-treated morphine-dependent animals. This indicates that MK-801 might be useful in inhibition of development of tolerance to antinociceptive effect of morphine [63]. Administration of another NMDA receptor antagonist memantine has also been shown to cause reduction in withdrawal jumping behaviour in morphine-dependent mice when administered 45 min prior to naloxone administration [61]. Similarly, kynurenic acid (a non-selective NMDAR antagonist at excitatory receptors) reduced the naloxone-precipitated withdrawal signs in rats. LY274614, which is a competitive antagonist of NMDA receptor was also reported to suppress the behavioural changes associated with morphine withdrawal [56]. However, NMDA antagonists have been shown to be less effective against the pre-existing disorder [61].

Role of PPARγ, NF-kB and inflammatory mediators

In CNS, PPARs are mainly related to lipid metabolism, and neural cell differentiation, cell death, inflammation and neurodegeneration [64]. PPARα and PPARγ are widely expressed in lateral hypothalamic region which are located in ventral tegmental area (VTA) that control dopamine release from VTA into Nucleus accumbens (NAc). These projections are associated with the reward pathway and have a potential role in addiction [65]. PPARγ belongs to the family of nuclear hormone receptors. They are ligand activated transcription factors, the function of which is to regulate the genes necessary for cell differentiation and different metabolic processes [66]. There are three types of PPAR isotypes, PPARα, PPARβ and PPARγ, distributed throug most of the peripheral tissues, neurons and glia in the brain [67]. Activation of these receptors decreases the TNF-α expression in adipose tissues and its inhibitory effect on insulin signalling. Monocytes and macrophages participate in inflammatory processes and their differentiation results in PPARγ expression. Morphological changes in the cell and Nitric oxide suppression result by activation of macrophages with PPARγ agonists. Activation of these nuclear receptors by agonists might induce apoptosis by interfering with NF-kB signalling pathway [68].

PPARγ agonists have been used for the mitigation of drug addiction in animal models. Thiazolidinediones (pioglitazone and rosiglitazone), have been shown to decrease alcohol consumption, alcohol seeking behaviour and withdrawal symptoms in animals [69]. PPAR receptor activation is also associated with attenuation in the expression of methamphetamine-induced locomotor sensitization [70]. A PPARα agonist inhibited the nicotine-induced DA release in the VTA-NAc projections, blocked nicotine seeking behaviour and decreased self-administration while prevented relapse in abstinent animals [71]. Along the same lines, PPARγ has been shown to play a crucial role in the morphine-related tolerance and dependence and interesting observations have been reported. Ghavimi et al. have shown that Pioglitazone ameliorated the symptoms of morphine tolerance and dependence in rats [72]. In their study, tolerance was developed by morphine over a period of 17 days whereas pioglitazone delayed the establishment of tolerance by 15 days. Interestingly, the positive effects of pioglitazone on morphine dependence and tolerance were reversed in the presence of GW-9662, a PPAR-γ antagonist. Chronic administration of morphine can induce a neuroinflammatory response characterized by the activation of glial cells and release of pro-inflammatory cytokines [73]. These inflammatory mediators can alter neuronal excitability and plasticity, reducing the effectiveness of morphine and contributing to tolerance. Neuroimmune interactions in morphine tolerance may also involve the activation of toll-like receptor 4 (TLR4) that are part of the innate immune system [74]. Morphine can bind to TLR4, triggering the release of pro-inflammatory cytokines and glutamate, which can increase morphine tolerance [73, 75]. Beneficial effect of pioglitazone on morphine tolerance and neuro-inflammation in the lumbar region of the rat spinal cord have also been demonstrated by Charkhpour et al. [76]. Similar results were observed in rat cerebral cortex where decreased neuroinflammation was linked with pioglitazone effects in morphine antinociceptive activity [77]. The effectiveness of SJP-005 (combination of ketotifen and ibuprofen) developed for the management of pain and opioid use disorder in reducing the morphine associated tolerance in rats has also been demonstrated [78]. Ibuprofen considered as PPARγ agonist reduced pain through the inhibition of cyclooxygenase pathway and production of prostaglandins, while Ketotifen, a mast cell stabilizer, H1-receptor antagonist and TLR4 inhibitor, decreases the pro-inflammatory cytokines and TNF-α [79]. Interestingly, development of morphine dependence was also observed with selective Phosphodiesterase-5 inhibitor (sildenafil) when given before the administration of pioglitazone. Sildenafil has been shown to increase the cGMP in rat dorsal hippocampus and enhance antinociceptive effect of morphine. In another study, immunohistochemical analysis indicated increased cGMP staining in neuronal region in hippocampus following treatment with sildenafil [80]. However, the role of Pioglitazone and PPARγ remains controversial as Javadi et al. have reported that the development of morphine dependence could be potentiated by these agents, possibly through NO/GC/cGMP pathway [8].

Depending upon tissue, receptor and pharmacodynamics of receptor activation, opioids might play neurodegenerative or neuroprotective roles within the CNS. Protective effect of morphine involves the activation of PI3K pathway that promotes cell survival and inhibits apoptosis by downstream signalling target Akt which activates NF-kB via phosphorylation of IkB kinase and helps in cell survival. NF-kB is also involved in the pathophysiological conditions related to CNS including stroke, brain trauma, spinal cord injury and epilepsy [81]. Opioids can also induce TNF-α which in turn activates macrophages and leads to cell proliferation, extracellular matrix remodelling and cytokine release consequently activating MAPK signal cascade. This series of steps further activates NF-kB and other transcription factors. TNF-α translocates NF-kB to nucleus causing increase in inflammatory cytokines in astrocytes. Morphine exerts oxidative stress in immune system and CNS that induces TNF-α and increases TNF-α expression in astrocytes, microglia and U937. Morphine exposure can also phosphorylate p38MAPK and its targets (Ask-1 and MKK4) which ultimately regulate various intracellular events including the expression of TNF-α. Hence, morphine can induce cascades of the biochemical intracellular signalling which up-regulate TNF-α and ROS and the functions of NF-kB and p38MAPK pathway [82]. Over-activation of NMDA receptors is also associated with the pathophysiology of opioid dependence and withdrawal where increase in activity of NMDA receptor activates NF-kB transcription. This crosstalk between NMDA and NF-kB may be involved in development of morphine dependence. In vitro studies have shown that NF-kB inhibition suppress naloxone-induced opioid withdrawal in isolated guinea pig ileum. It was observed that the administration of DDA (diethyl dithiocarbamic acid sodium salt trihydrate), a selective NF-kB inhibitor, attenuated the development of morphine dependence observed through withdrawal signs like jumping, rearing, circling behaviour and fore paw licking [81].

A summary of the molecular mechanisms involved in morphine-induced tolerance and dependence has been summarized in Fig. 1 while findings in some of the key studies have been presented in Table 1.

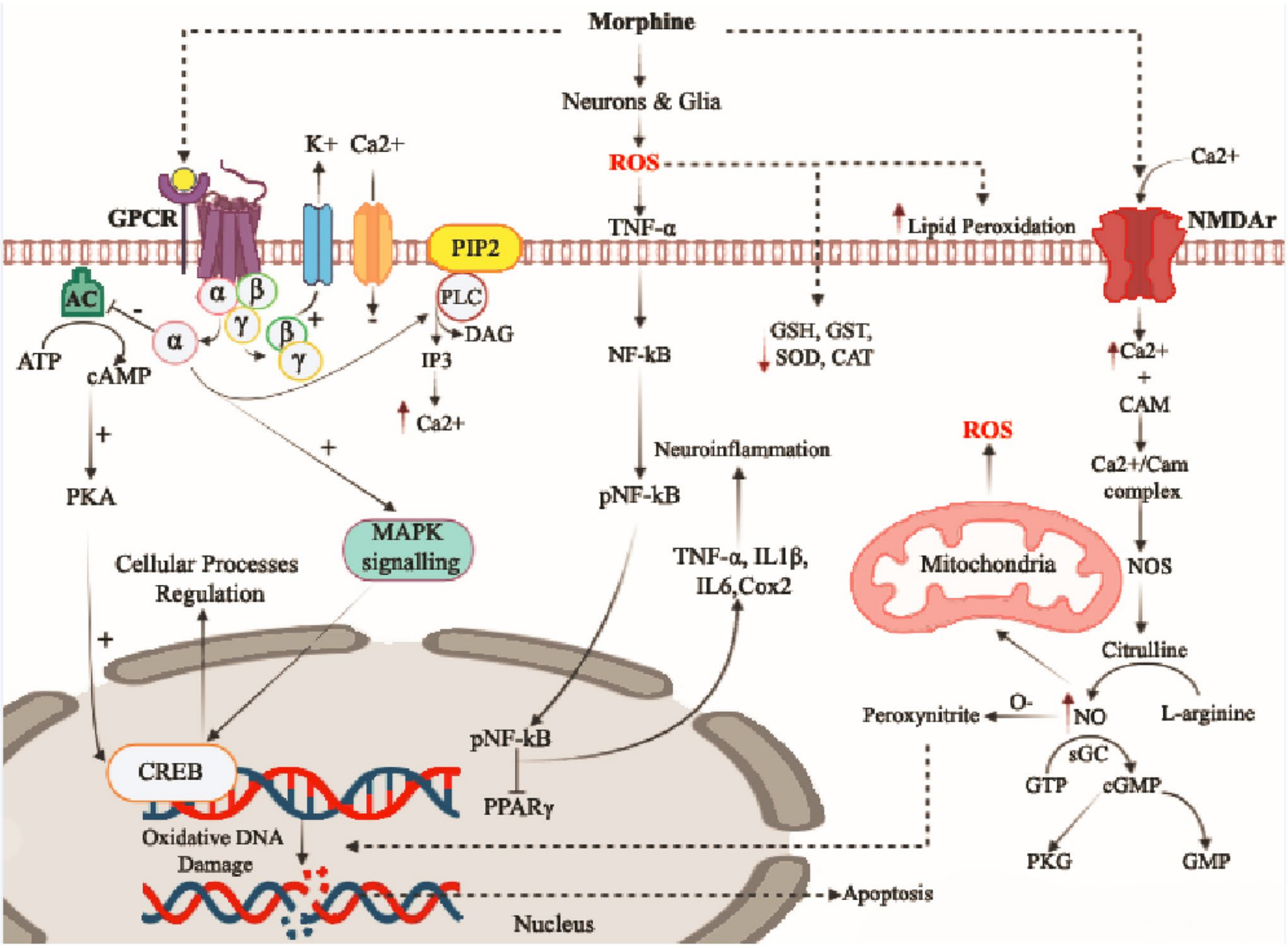


Fig. 1 A schematic diagram showing multiple downstream signalling pathways that mediate morphine-induced tolerance and dependence. Chronic morphine exposure phosphorylates opioid receptors leading to inhibition of adenylate cyclase activity and ultimately decreasing cAMP level and protein kinase A. This leads to potassium channel activation and cellular hyperpolarization. Morphine-induced activation of Phospholipase C increases inositol-(1,4,5)-triphosphate (IP3) and 1,2-diacylglycerol (DAG) resulting in increase in the level of calcium inside the cell. Phospholipase C is strongly associated with morphine activity. The antinociceptive effect is potentiated and tolerance is reduced through inhibition of phospholipase C with a single dose of morphine [20]. On the other hand NMDA receptor and its downstream signalling cascades is believed to have a vital role in different forms of experience-dependent changes in brain and behaviour [136]. NMDA receptor activation leads to rise in calcium level in post-synaptic neuron. Calcium then binds to calmodulin which results in nitric oxide (NO) formation through activation of nitric oxides synthase (NOS). NO formed activates soluble guanylate cyclase (GC) and increases the formation of cGMP. Blocking any step in the pathway prevents neuronal damage [137]. The excessive NO produced can damage mitochondria through nitrosation. Mitochondria and their metabolic processes are effected by reactive species and results in rise in the level of Reactive oxygen species (ROS) which in turn result in oxidation of DNA, lipids and proteins [138]. The μ-opioid receptors also activates MAPK-signalling cascade, which leads to NF-kB activation and its translocation to the nucleus. Through the recruitment of NF-kB pathway, morphine induces oxidative stress through its impact on ROS and TNFα pathways [82]

Behavioural sensitization in morphine associated dependence

Behavioural sensitization refers to intensified behavioural or motor response occurring after repeated exposure to certain abusive substances in drug-free environment. Sensitization may aggravate the dependency on drug when the individual confront some stimuli associated with drug causing cravings. The environment plays a pivotal role where the addictive substance is utilized. Psychological addiction can be evaluated by studying behavioural sensitization. Research studies on animal models demonstrate that this behaviour after cessation or diminished use of drugs and or cues might lead to relapse [83]. The increase in rewarding effect of an addictive substance can also play part in the relapse of drug use. In animal models, such behaviour might be accessed through experimental behavioural protocol known as conditioned place preference test [84, 85].

The progression of behavioural sensitization involves neuroadaptive changes involving dysregulation in dopaminergic

Table 1 Summary of key research studies on the molecular mechanisms of morphine dependence and tolerance

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
1	TNF alpha production in morphine-treated human neural cells is NF-κB-dependent [82]	Morphine-induced inflammation	Human monocyte cell line U937, Primary astrocytes and microglia	NF-kB, TNFα	Cell culture, transfection and infection assay	PCR, Western Blot,	Morphine increases expression of TNFα in U937, astrocytes and microglia and is involved in translocation of NF-kb to nucleus
2	Nuclear factor-κ-B inhibitor modulates the development of opioid dependence in a mouse model of naloxone-induced opioid withdrawal syndrome [81]	Morphine dependence	Swiss albino mice (25 + -2 g)	N-kB / To check the effect of DDA (selective NF-kB inhibitor)	Morphine IP 5 mg/kg two times a day for 5 days. On the last day, 8 mg/kg naloxone (IP.) is given after which Behavioural observations were made (30 min)	Morphine withdrawal symptoms (Jumping, WSS, rearing, forepaw licking, circling behaviour)	DDA attenuated the morphine-induced dependence which shows the involvement of NF-kB. DDA has no effect on CNS which was assessed by locomotor activity
3	The NMDA antagonist memantine blocks the expression and maintenance of morphine dependence [61]	Morphine dependence	Mice (29-32 g)	NMDA /To check the effect of memantine (7.5–30 mg/kg IP)	Morphine (30 mg/kg) twice daily (3 days and last dose only in morning on 4th day). Memantine was administered to one of the group. Number of jumps were recorded after naloxone injection (4 mg/kg)	Morphine withdrawal symptoms (jumping), Locomotor activity (EYE and TRACK-ANALYZER program)	Memantine reduces expression of naloxone-precipitated morphine withdrawal While Glycine reversed memantine-induced reduction in naloxone precipitate jumping
4	Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801 [63]	Morphine dependence and tolerance	Rats	NMDA pathway. The effect of MK-801 (NMDA inhibitor) was studied	Morphine 10 mg/kg. The experiment was conducted for 10 days	Behavioural test for tolerance (Tail-flick test), Morphine dependence (Number of jumps)	NMDA receptors has significant role in development of opioid dependence and tolerance MK-801, NMDA receptor inhibitor (0.03 mg/kg) reverse tolerance development

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
5	Role of nitric oxide in the induction and expression of morphine tolerance and dependence in mice [54]	Morphine-induced dependence and tolerance	Male Swiss albino mice (25–30 g)	Nitric oxide pathway, Inhibitors used (D-NAME, L-NAME, L-NMMA) 6Precursor used (L-arginine)	Morphine twice daily for 2 days and on 3rd day assessment was performed	Tolerance test (Tail-Flick test), Dependence test (jumping behaviour and wet dog shakes)	L-arginine delayed development of Morphine dependence and tolerance. L-ARG 'induces' tolerance in opioid naive mice through NO production, and increases tolerance when given with morphine while NOS inhibitors inhibits morphine tolerance
6	The nitric oxide–cGMP signaling pathway plays a significant role in tolerance to the analgesic effect of morphine [58]	Morphine-induced Tolerance	Male Wistar albino rats (180–210 g)	Nitric oxide and cGMP pathway	Subcutaneous dose of 50 mg/kg morphine (Once a day) was used to induce tolerance from day 1 through day 3. On day 4, tail-flick and hot-plate tests was performed, then subcutaneous dose of 5 mg/kg of morphine was injected as a challenge dose and after 15 min, tail-flick and hot-plate tests were carried out, to average them so that to evaluate the post-drug latency and tolerance development to morphine	Behavioural test (Hot-plate test and tail-flick test)	NO–cyclic GMP system activation by the NO-independent activators enhances the development of morphine antinociceptive tolerance. L-NAME significantly reduces the development of tolerance to morphine

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
7	Hyperbaric oxygen attenuates withdrawal symptoms by regulating monoaminergic neurotransmitters and NO signaling pathway at nucleus accumbens in morphine-dependent rats [57]	Morphine dependence	Male SD rats (180-220 g)	Nitric oxide pathway. Effect of hyperbaric oxygen (HBO2) in morphine withdrawal	Following acclimation for 1 week, the rats were separated into 4 groups Group 1 (normal control) Received physiological saline . Groups 2–4 (morphine) at increasing doses (20, 40, 60, 80, 100, 100 and 100 mg/kg twice daily for 7 days) Group 3 (positive control) Clonidine injected (0.4 mg/kg intragastrically) once per day 30 min prior to the morning morphine injection. Group 4 (HBO2 Group) HBO2 Treatment was given 30 min before the morning morphine injection	Assay of NO and NOS in the NAc, Measurement of the Contents of DA, NE and 5-HT Contents (HPLC) , Determination of cGMP and cAMP Levels in NAc, (Immunohistochemistry)	HBO2 Effectively attenuated withdrawal symptom and weight loss HBO2 normalized the NO, NOS, cAMP and cGMP levels in morphine-dependent rats Results shows that HBO2 mitigation of morphine withdrawal symptoms involves regulation of monoaminergic neurotransmitters and NO–cGMP pathway

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
8	Modulation of the NO/CO-cGMP signaling cascade during chronic morphine exposure in mice [59]	Morphine tolerance	Mice C57BL/6	NO/CO-cGMP	Morphine 75 mg/kg pellet were used. Mice were briefly anesthetized and a small subcutaneous skin pocket was 9made on the animal's back into which a morphine tablet was placed (uncoated Side down) followed by closure with surgical staples. Animals in the control group had a skin pocket made and had Their incisions closed. 6 days after surgery lumbar spinal cord segments (L3–6) were dissected following sacrifice by CO2	RT-PCR, Western blot	The CO/NO-cGMP pathway is up regulated at multiple Points after morphine exposure indicating a coordinated molecular and biochemical response

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
9	Pioglitazone potentiates development of morphine dependence in mice: Possible role of NO/cGMP pathway [8]	Morphine dependence	Mice, human glioblastoma (U87) cell line	NO/cGMP pathway, PPARγ pathway. Effect of pioglitazone on morphine dependence	Morphine i.p. three Times daily at 08.00, 11.00 and 16.00 hat the doses of 50, 50 and 75 mg/kg, respectively, Morphine administration was carried out over 4 days for all Groups of mice. Morphine 100 mg/kg was injected on (5th) day. Naloxone (4 mg/kg, i.p.) was injected in order to induce withdrawal signs	RT-PCR, Cell culture studies, cAMP and cGMP assay, evaluation of mRNA expression in U87 cells. In vitro culture studies	Pioglitazone potentiated naloxone-induced withdrawal syndrome in morphine-dependent mice *in-vivo*. While inhibition of PPARγ, nNOS or GC could reverse the pioglitazone-induced potentiation of morphine withdrawal signs. Sildenafil, amplified its effect. Nitrite levels in the hippocampus were significantly increased in pioglitazone-treated Morphine-dependent mice. In the human glioblastoma (U87) cell line, rendered dependent to morphine, cAMP levels did not show any alteration after chronic pioglitazone administration While cGMP measurement revealed a significant rise Pioglitazone has the ability to increase morphine dependence and to augment morphine withdrawal signs. The possible pathway underlying this effect is through activation of NO/GC/cGMP pathway

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
10	Analgesic tolerance to morphine is regulated by PPAR γ [133]	Morphine Tolerance	C57 mice (28-30 g), KO mice having neuron-specific PPARγ deletion and WT counterparts	PPARγ	Morphine given two times daily for 9 days. On the first and last day of the experiment, dose was reduced of morphine injected (15 mg·kg – 1). For the other 7 days of the experiment, the morphine dose was 30 mg·kg – 1 (in Exp. 1, 2 and 4) or 15 mg·kg – 1 (in Exp. 3 and 5). In certain experiments, mice received pioglitazone (0, 10 or 30 mg·kg – 1) and/or GW-9662 (0, 2.5 or 5 mg·kg – 1) before the morphine injections. For the evaluation of the effects of pioglitazone on reversal of morphine tolerance, animals received pioglitazone only in the evening of day 8 and on day 9, before the morphine injections Analgesia was monitored on alternate days	Tail immersion test, locomotor activity	Effect of pioglitazone was not present in KO mice lacking the PPAR. Facilitation of the development of analgesic tolerance to morphine in these mice, which further confirms the role of neuronal PPARγ in the regulation of this phenomenon. Therefore, the neuron-mediated mechanisms are responsible for the effects of pioglitazone However, an indirect role of glia-mediated mechanisms that could be under the control of neuronal PPARγ cannot be excluded. Further studies are needed in order to clarify this point Results indicate that PPARγ shows an important role in modulating tolerance to morphine, and pioglitazone may potentially be used as an add-on medication to attenuate the development of opioid tolerance when these drugs are used for chronic pain

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
11	Pioglitazone attenuates the opioid withdrawal and vulnerability to relapse to heroin seeking in rodents [79]	Opioid dependence and relapse	Male Wistar rats and CD1 mice	PPARγ	10, 30, or 60 mg/kg p.o. PIO (PPAR-) Single treatment 1 h prior to morphine injection the evening of day 5 and morning of day 6 (withdrawal expression) Treatment twice daily (12 h and 1 h prior to tests) for 5 consecutive days, then again on the morning of day 6 1 h prior to final morphine injection (withdrawal development) Two treatments, 12 h and 1 h prior to reinstatement tests	Morphine withdrawal (jumps, paw tremors, teeth chattering, and wet dog shakes) Heroin seeking and relapse (stress-, cue-, and heroin-induced reinstatement)	In mice, PIO (10 and 30 mg/kg) attenuated the expression of morphine withdrawal and the development of morphine withdrawal (at 30 mg/kg); pre-treatment with 5 mg/kg GW9662 (PPAR-antagonist) reversed the effect of PIO on expression of withdrawal In rats, PIO significantly reduced yohimbine-induced reinstatement (at 30 mg/kg, but not 10 mg/kg) and heroin-induced reinstatement (at 30 mg/kg and 60 mg/kg, but not 10 mg/kg) of heroin-seeking, but had no effect on cue-induced reinstatement (at 10, 30, or 60 mg/kg)

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
12	Lung opioid receptors: pharmacology and possible target for nebulized morphine in dyspnea [7]	Morphine dependence	Male NMRI mice (25–29 g) Human glioblastoma cells T98G	P13K/Akt and NO pathway	Morphine was given 3 times a day (50, 50 and 75 mg/kg for 4 days). The last high dose (100 mg/kg) reduced the overnight withdrawal. On the 5th day 100 mg/kg of morphine was injected followed by 1-h later IP injection of naloxone (4 mg/kg)	Morphine withdrawal signs (jumping, Diarrhea, weight loss and grooming) Quantification of nitrite in hippocampus Cell toxicity and micro culture tetrazolium test assay qRT-PCR Western Blot	Effect of thalidomide on morphine dependency at different doses (2.5–15 mg/kg, i.p) was evaluated by administering it 45 min prior to each dose of morphine for 5 days Thalidomide inhibits PI3K/Akt and NO pathway in order to attenuate the development of morphine dependence Thalidomide (15 mg/kg) has significantly inhibited the development of morphine dependence Thalidomide and/or PI3K and/or Akt inhibitor pre-treatment inhibited induction of iNOS by morphine in T98G cells

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
13	Effect of suvorexant on morphine tolerance and dependence in mice: role of NMDA, AMPA, ERK and CREB proteins [62]	Morphine dependence and tolerance	adult male Razi mice weighing 25 – 30 g	NMDA, AMPA, ERK and CREB proteins	Morphine was injected subcutaneously 3 times daily to mice (50, 50, and 75 mg/kg) for 3 days. A single dose of morphine (10 mg/kg) was injected on the first day (before the start of the infusion) and the fourth day	Morphine withdrawal symptoms: Jumping Tolerance test: Tail-flick test Locomotor activity: Open-field test Western Blot	Results of this study show that repeated administration of morphine-induced tolerance and dependence through elevation of NMDA, p-ERK, and CREB proteins levels in mice brain. On the other hand, suvorexant reduced morphine-induced tolerance and dependence. Suvorexant appears to exert its effects on the prevention of opioid tolerance and dependence through blocking orexin receptors and reduction of p-ERK and CREB protein levels in brain

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
14	Involvement of the nociception opioid peptide receptor in morphine-induced antinociception, tolerance and physical dependence in female mice [134]	Morphine tolerance and dependence	Female C57BL/6 J mice, 8 weeks old, weighing 18–20 g In this study, a selective agonist (SCH221510) and antagonist (SB612111) of the NOP receptor were used	Nociceptin opioid peptide (NOP) receptor	Tolerance: Antinociceptive tolerance was induced by injecting mice with morphine (10 mg/kg, sc) twice daily for six days Antinociception test was performed every day Dependence: Morphine was injected (10, 20, 30, 40 and 50 mg/kg) two times daily (five days). Naloxone (5 mg/kg, ip) was injected 4 h after the last dose and withdrawal symptoms were recorded The effect of SCH221510 (3 mg/kg, sc) or SB612111 (10 mg/kg, sc) was also studied in tolerance and dependence model	Tolerance test: Hot-Plate test Locomotor activity Rotarod test	Taken together, this study found that systemic activation of NOP receptors attenuated morphine antinociception to acute thermal stimuli, facilitated the development of morphine-induced antinociceptive tolerance and did not robustly alter the morphine-induced physical dependence in female mice. Systemic blockade of NOP receptors produced opposite actions. These results demonstrate that the endogenous N/OFQ-NOP receptor system plays diverse roles in modulating the pharmacological profiles of μ-opioid receptor agonists

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
15	AG protein signaling-biased agonist at the µ-opioid receptor reverses morphine tolerance while preventing morphine withdrawal [101]	Morphine Tolerance	222 male and 16 female C57BL6 mice	µ-opioid receptors	Mice were dosed cumulatively with morphine (6–48 mg/kg, i.p., salt weight) or SR-17018 (6–48 mg/kg, i.p., base weight) after the chronic treatments Immediately after Recording baseline responses, mice were injected with the first dose. After 1 h, mice were again tested and injected with the next highest dose	Hot-plate test	We show that SR-17018, a compound that shows a strong preference for G protein signaling over ßarrestin2 recruitment in cellular assays, produces less tolerance than morphine we show that unlike morphine, chronic treatment with SR- 17,018 does not induce MOR desensitization Substitution of SR-17018 in morphine-tolerant mice restores morphine sensitivity within 3 days

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
16	Mitragynine attenuates morphine withdrawal effects in rats—a comparison with methadone and buprenorphine [135]	Morphine withdrawal	Male Sprague–Dawley rats, (weight 200–300 g)	μ-opioid receptors	Based on the morphine withdrawal model, rats were morphine treated with increasing doses from 10 to 50 mg/kg twice daily over a period of 6 days. The treatment was discontinued on day 7 in order to induce a spontaneous morphine abstinence. The withdrawal signs were measured daily after 24 h of the last morphine administration over a period of 28 abstinence days. In rats that developed withdrawal signs, a drug replacement treatment was given using mitragynine, methadone, or buprenorphine and the global withdrawal score was evaluated	Withdrawal behaviours were distinguished as "counted signs," including chewing, head shakes, exploring, digging, yawning, teeth chattering, wet dog shakes, writhing and as "checked signs," including squeaking on touch, hostility on handling, and diarrhea	The morphine withdrawal model-induced profound withdrawal signs for 16 days Mitragynine (5–30 mg/kg; i.p.) was able to attenuate acute withdrawal signs in morphine-dependent rats. On the other hand, smaller doses of methadone (0.5–2 mg/kg; i.p.) and buprenorphine (0.4–1.6 mg/kg; i.p.) were necessary to mitigate these effects

Table 1 (continued)

S. No	Title/author	Study design/model	Species/strain	Molecular pathway/target study	Dose/route of administration/disease induction	Treatment regimen/behavioural studies/molecular studies	Primary findings
17	Thalidomide attenuates the development and expression of antinociceptive tolerance to μ-opioid agonist morphine through l-arginine-iNOS and nitric oxide pathway [15]	Morphine tolerance	Male NMRI mice having weighing of 22–25 g	Nitric oxide pathway T98G human glioblastoma cell	In order to induce tolerance to antinociceptive effect of morphine, mice were treated repeatedly with i.p injection of morphine three times (50, 50 and 75 mg/kg) a day for 4 consecutive days. On 5th day only a single dose (50 mg/kg) of morphine was injected The development of antinociceptive tolerance was measured throughout for 5 days after 1st injection of morphine on each day while expression phase was studied on 5th day using hot-plate test and Tail -Flick test	Hot-plate test Tail-flick test	Thalidomide has protective effect both in the development and expression phases of morphine antinociceptive tolerance. It is also evident that this effect of thalidomide is induced by the inhibition of NOS enzyme predominantly iNOS

and glutamatergic neurotransmissions [86, 87]. The drug-induced neuroadaptations is linked to the dopaminergic "reward" circuitry [88]. Much of the attention in drugs of abuse is given to the mesolimbic dopamine pathway that includes dopaminergic neurons in the VTA residing in the midbrain and their projections into the limbic forebrain, into the NAc [89]. Dopaminergic pathway involves VTA and glutamatergic pathway involves prefrontal cortex, both ending in NAc [87]. Interestingly, hippocampus is anatomically positioned in such a way that it also influences the reward circuitry of the brain, receiving inputs from NAc and VTA and sending inputs to the NAc. These neuroanatomical connections influence dopamine in the NAc, VTA dopaminergic cell firing and dopaminergic behaviour [88]. Morphine mediates efflux of dopamine in the nucleus accumbens indirectly via binding to the MORs [88]. Morphine sensitization is linked with augmented release of dopamine in the mesolimbic area having dopaminergic D1 receptors [90]. Impairment in sensitization has been observed experimentally by blocking the D1 receptors. Additionally, previous research data have demonstrated association of increased MAP kinase activity (ERK 1/2) with enhanced expression of D1 receptors during morphine sensitization, which is decreased by the application of D1 receptor antagonist [91]. In contrast, behavioural sensitization was developed in association with glutamatergic pathway in VTA, as NMDA and AMPA receptor antagonist inhibited the acquisition of behavioural sensitization but did not affect its expression [92, 93].

Current treatment approaches and future perspectives

Currently, clonidine, methadone and naltrexone are used to treat opioid dependence and withdrawal symptoms but they have side effects and have higher chances of relapse [57]. Individual who undergo treatment must have 10 days of abstinence period called the "induction hurdle" after naltrexone injection. During this period, the withdrawal symptoms are severe and the individual may not comply with the treatment regimen which increases the chances of relapse. Supportive therapy may also be helpful in decreasing the severity of the withdrawal symptoms. These medications include non-steroidal anti-inflammatory agents like ibuprofen, antihistamines, anti-emetics such as ondansetron and anti-diarrheals like loperamide. Various trials have shows the effectiveness of lofexidine and clonidine in modifying opioid withdrawal symptoms. The adverse effects include sedation, drowsiness, dizziness, dry mouth and hypotension [34]. Methadone, a synthetic opioid is usually utilized as a substitution therapy for opioid. It has a longer half-life as compared to morphine, which aids in managing opioid withdrawal symptoms [94, 95]. However, methadone like all MOR agonists carries its own potential for dependence and addiction [96]. Methadone exhibits a unique pharmacological profile; it acts as an antagonist at NMDA receptors and also blocks certain monoamine reuptake transporters, enhancing its analgesic effect and reducing tolerance [97, 98]. Buprenorphine, a partial agonist for MOR may be used alone and in combination with other μ-opioid receptor antagonist like naloxone in the treatment of opioid withdrawal. Clonidine, an α_2-adrenergic receptor agonist is also used in opioid withdrawal. It targets noradrenergic hyperactivity in locus coeruleus neurons that is associated with opioid withdrawal symptoms but may cause side effects like hypotension and sedation. Lofexidine (structural analogue of clonidine) is another α_2-receptor agonist with mild side effects. Lofexidine was found to be efficacious in the treatment of morphine withdrawal with tolerable side effects but it could not completely subside withdrawal symptoms [99]. Side effects are more common with α_2-adrenergic agonists as compared to methadone [100]. Hence, more innovative drugs and strategies are needed to overcome these problems.

Effects of Thioredoxin-1 (Trx-1) on morphine withdrawal and relapse provides another area of research in morphine associated tolerance and dependence [52]. New compounds might be synthesized like SR-17018 which has distinctive capability to preferentially stabilize the MOR by bringing conformational change at G proteins. This unique conformational change confers significant analgesic efficacy, particularly during states of opioid dependence. Remarkably, SR-17018 achieves this therapeutic benefit while attenuating the severity of withdrawal symptoms. Importantly, it also promotes the recruitment of β-arrestin, which is detrimental as many of the side effects of opioids such as respiratory depression, constipation, and tolerance are associated with this mechanism. Therefore, SR-17018 presents a compelling case for the development of next-generation analgesics, as it not only stabilizes MORs, but also potentially restores their sensitivity, offering a promising path towards improved opioid therapies. [101]. Thalidomide, a glutamic acid derivative, has also been evaluated for its neuroprotective role in morphine dependence and tolerance. Thalidomide exhibited promising effect in both development and expression phases by inhibiting iNOS [15]. SR-17018, a compound having affinity for MORs has shown effectiveness in suppressing signs of the withdrawal, and inhibiting the development of antinociceptive tolerance. SR-17018 was also found effective in morphine tolerant mice where it significantly restored sensitivity to morphine within three days. Moreover, SR-17018 treated animals exhibited less tolerance as compared to morphine and oxycodone [101].

In recent years neurons and glial cells lost in the neurological disorders have successfully been regenerated from stem cells such as neural, embryonic and mesenchymal stem cells [102]. Expectations regarding mesenchymal stem cell (MSC) therapy are high [103]. MSCs can be isolated from bone marrow, muscles, fetal liver, lung, adipose tissue and umbilical cord and can be expanded in-vitro [104]. Evidence indicates that the MSCs are suitable for long-term culturing in-vitro, without any effect on their morphology, function, karyotype and phenotype [105]. Li et al. evaluated MSC therapy in prevention and reversing opioid tolerance (OT) and opioid-induced hyperalgesia (OIH) in rat and mouse model. Modulating neuroinflammation can be plausible target in treatment, as it is linked with the development of OT and OIH. Intrathecal and intravenous mesenchymal transplantation (MSC-TP) prevented and showed therapeutic effects in both OT and OIH [106]. Significant reversal of OT and OIH was observed with MSC-TP performed before injection of morphine and no abnormality in the vital organs or functions was observed in any of the animal [107]. Further studies indicated that the bone marrow-derived human mesenchymal stem cells (hMSCs-BM) reduced morphine tolerance in a dose-dependent manner. These hMSCs-BM have been shown to inhibit morphine-induced up-regulation of cAMP in retinoic acid-differentiated SH-SY5Y cells [108]. The hMSCs-BM can be used for the treatment of opioid tolerance and dependence and also can reduce the risk of drug abuse and addiction [108]. Despite the applications of MSC transplantation; a lot of potential risks are there such as pro-tumorigenic, immune response, undesirable differentiation, short survival rate, no improvement, unspecified optimal doses and route of cell administration [102, 103, 109]. This far, the use of MSCs therapies in various preclinical and clinical studies has been accomplished and is a controversial breakthrough in the treatment and amelioration of the incurable diseases [105]. For genetic manipulation and gene transfer into the central nervous system, *ex-vivo*, immortalized human neural stem cells are considered to be the most efficient as they survive well, have better integration with host tissues and also differentiate into both neuronal and glial cells [102]. The extrinsic and heritable intrinsic signals play vital role in giving rise to the cellular diversity in the CNS. Therefore, further studies are needed for the identification of the signal molecules or regulatory genes for neural stem cell proliferation, differentiation and integration and determination of the favourable conditions for its implantation to survive, prosper and restore the damaged part of the brain [102]. Embryonic stem cells or neural stem cell-derived neurons and glial cells can be the renewable source of cells in cell-based therapy for neurological disorders. The limitations of the cell-derived neurons or glial cell are due to the uncertainty of long-term survival and the phenotypic stability of these cells in a graft after transplantation, the unpredictable interactions amongst the grafted cells or interaction with the host neurons due to the presence of unwanted neurons or glial cell types in a purified sample and the formation of tumor in the graft site after transplantation [102].

The discovery of electrical activity in human brain has led to many scientific breakthroughs, having diverse range of applications in clinical and cognitive neurosciences [110]. These techniques have gained importance in the field of neurosciences providing the information about where and when reward circuitry is altered [111]. EEG procedure involves temporal resolution performed in milliseconds and gives dynamic balance between inhibition and excitation in neural networks of brain and are valuable method for studying sensory, motor and cognitive phenomena [110]. EEG is used to study the neural activity presenting in the form of local field potentials, somatic action potentials, calcium spikes, flow of current through ion channels and reverse propagating action potentials [112]. The brain oscillations reflect the functioning and dysfunction of the brain [113]. EEG studies may help identify individuals having substance use disorders during rational decision making [111]. Drug cue reactivity paradigms together with EEG recordings can be used in identifying the ones at risk of developing substance use disorder and to predict the chances of relapse for those in recovery [111]. In abstinent addicts, behavioural, cellular, molecular and EEG studies can identify reduction in the activity in the projections evolving from prefrontal cortex into the basal ganglia [114]. By measuring the behaviour and changes in the neuronal activity associated with rostral ventromedial medulla (RVM), tolerance was assessed. EEG measures were performed in order to evaluate the loss of morphine inhibition of nociception, concluding that the development of tolerance was through direct action of morphine in the neurons associated with ventrolateral periaqueductal gray (vPAG) [115]. In another study, the EEG evidence revealed that acute morphine administration disrupted the balance between excitation and inhibition of VTA neurons, targeting the GABAergic tail of the VTA/ rostromedial tegmental nucleus (RMTg) [116]. EEG results indicate that chronic administration of morphine is associated with an in increase in VTA-DA neuronal discharge and bursting in naïve animals. Adaptive changes such as development of tolerance and hyperactivity in VTA-DA neurons are also related to the morphine use [117]. The effect of chronic morphine administration on dopamine in the pyramidal cells of basolateral amygdala cells was conducted by Li et al. using whole-cell patch-clamp method showing the transmission of dopamine on excitatory synapse from inhibition to excitation and its action on dopamine 1 (D1) receptors [118]. The patch-clamp technique provides a high-resolution recording of changes in the ionic current flowing through plasma membrane of the cell [119]. Whole-cell patch-clamp electrophysiology of neurons is considered to be the gold standard technique for the analysis of molecular mechanisms underlying neural computation and pathology. Automated technique using patch clamping in-vivo have demonstrated good outcomes and quality of intracellular recordings in cortex and hippocampus in mouse [120]. EEG studies have also helped in understanding the complex nature of the brain signalling.. Although, methodologies such as functional magnetic resonance imaging (fMRI), electrophysiology (EEG) and positron emission tomography (PET) are available, EEG is preferred over the others as it is economical, portable and user friendly [111].Patch clamp technique is the default method used today for studying cellular electrophysiology. The limitation of this technique is the art required for its use which is challenging as the target such as the biological structures becomes smaller and smaller. Nonetheless, this technique has a tremendous impact in the area of life sciences temporally in parallel with the advancements in the field of nanoscience.

The development of bifunctional and bivalent analgesics presents an innovative approach in the management and treatment of chronic pain, particularly for the ongoing quest to eliminate dependence and tolerance associated with opioid drugs. This multifaceted strategy aims to engineer compounds that can target more than one type of receptor or signalling pathway to potentiate analgesic effects while minimizing adverse outcomes such as dependence and tolerance [121]. Bifunctional analgesics are single molecules that interact with multiple targets. One of the most promising areas in this space is the development of MOR agonist/DOR antagonist molecules. Preclinical evidence suggests that such bifunctional MOR/DOR drugs might attenuate the development of tolerance and dependence typically associated with pure MOR agonists, while maintaining strong analgesic effects [122, 123]. Bivalent analgesics consist of two linked pharmacophores that can simultaneously engage different sites on the same or different receptors. A notable example is the bivalent ligand MDAN-21, which shows high affinity and selectivity for MOR and the nociceptin/orphanin FQ peptide (NOP) receptor. Studies have shown that MDAN-21 exhibits potent analgesic properties in models of acute and chronic pain without inducing tolerance or physical dependence [124, 125]. Moreover, emerging research indicates that bifunctional and bivalent analgesics may also have a role in managing opioid-induced hyperalgesia, a condition where long-term opioid usage paradoxically increases pain sensitivity. The MOR/NOP bifunctional compound AT-121, for example, has been shown to provide potent analgesia without producing hyperalgesia, even after chronic administration [126].

Mitragyna speciosa is a tropical tree native to Southeast Asia and is known as “kratom”. Its leaves are highly rich in indole alkaloids, including mitragynine, having potential as multitarget analgesic [127]. It is well-established that mitragynine acts as partial agonist of MOR, with lower affinity compared to conventional opioids like morphine. However, its unique pharmacological profile extends beyond MOR, as it also interacts with other targets, including kappa and delta opioid receptors, adrenergic, serotonergic, and dopaminergic receptors, making it a multitarget analgesic. Interestingly, recent studies suggest that mitragynine metabolite, 7-hydroxymitragynine, has a higher affinity and efficacy at MOR, contributing significantly to the analgesic effects of kratom [128]. However, unlike traditional opioids, mitragynine and its metabolites appear to recruit less β-arrestin, a protein involved in receptor desensitization and downregulation, which could potentially lead to fewer side effects such as respiratory depression and constipation [129]. The multitarget nature of mitragynine and its unique signaling profile might help mitigate the development of tolerance and dependence [130]. Furthermore, anecdotal evidence and preliminary scientific reports suggest that *M. speciosa* might aid in opioid withdrawal, though more rigorous clinical studies are required to substantiate these claims [131]. The research on mitragynine and other indole alkaloids in *M. speciosa* is promising, however, there remain challenges to overcome, such as the lack of standardized products and the potential for misuse and toxicity. Nonetheless, these compounds represent an interesting avenue for the development of novel multitarget and multifaced analgesic drugs. It is expected that bifunctional and bivalent analgesics might result in safer and more effective pain management [132].

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Declarations

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