Published in final edited form as:
Curr Drug Targets. 2012 February ; 13(2): 230–246.

Current Research on Opioid Receptor Function

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Abstract

The use of opioid analgesics has a long history in clinical settings, although the comprehensive action of opioid receptors is still less understood. Nonetheless, recent studies have generated fresh insights into opioid receptor-mediated functions and their underlying mechanisms. Three major opioid receptors (μ-opioid receptor, MOR; δ-opioid receptor, DOR; and κ-opioid receptor, KOR) have been cloned in many species. Each opioid receptor is functionally sub-classified into several pharmacological subtypes, although, specific gene corresponding each of these receptor subtypes is still unidentified as only a single gene has been isolated for each opioid receptor.

In addition to pain modulation and addiction, opioid receptors are widely involved in various physiological and pathophysiological activities, including the regulation of membrane ionic homeostasis, cell proliferation, emotional response, epileptic seizures, immune function, feeding, obesity, respiratory and cardiovascular control as well as some neurodegenerative disorders. In some species, they play an essential role in hibernation. One of the most exciting findings of the past decade is the opioid-receptor, especially DOR, mediated neuroprotection and cardioprotection. The up-regulation of DOR expression and DOR activation increase the neuronal tolerance to hypoxic/ischemic stress. The DOR signal triggers (depending on stress duration and severity) different mechanisms at multiple levels to preserve neuronal survival, including the stabilization of homeostasis and increased pro-survival signaling (e.g., PKC-ERK-Bcl 2) and anti-oxidative capacity. In the heart, PKC and KATP channels are involved in the opioid receptor-mediated cardioprotection. The DOR-mediated neuroprotection and cardioprotection have the potential to significantly alter the clinical pharmacology in terms of prevention and treatment of life-threatening conditions like stroke and myocardial infarction.

The main purpose of this article is to review the recent work done on opioids and their receptor functions. It shall provide an informative reference for better understanding the opioid system and further elucidation of the opioid receptor function from a physiological and pharmacological point of view.

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Keywords

Opioids; opioid receptors; neurotransmitters; function; brain; heart; lung; ionic homeostasis; neuroprotection; hibernation; pain; hypoxia; ischemia

1. INTRODUCTION

The use of opioid analgesics has a long history, dating back several millennia. Despite the flow of time and the energies of scientists around the world, however, the function of opioid receptors in the brain is not well understood until this day. With the limited availability of data, controversies and diametrically opposite results are inevitable. For example, *in vivo* studies [1–4] showed that opioid receptor inhibition by opioid antagonists, such as the non-selective morphinan naloxone, protected the brain from ischemia-induced injury, while other studies [5–7] suggested that opioid receptor activation with opioid agonists were protective or extended animal survival time during severe hypoxia.

Recent studies utilizing current approaches have produced new information on opioid receptor-mediated function and their underlying mechanisms. For instance, our extensive studies specifically dissected out the role of DOR in neurons under hypoxic/ischemic conditions and demonstrated that the activation of DOR is neuroprotective against hypoxic/excitotoxic insults to cortical neurons, while its inhibition causes neuronal injury [8–16, 17]. Also, many other studies have recently demonstrated that DOR activetion is indeed neuroprotective against hypoxic/ischemic stress [18–31]. Besides opioid-receptor mediated neuroprotection, many new studies provide considerable evidence on involvement of opioid receptors in a multitude of functions throughout the body.

The main purpose of this article is to review the recent work done on opioids and functioning of their receptors under physiological and pathophysiological conditions. For the reader's convenience, we have briefly summarized the background information on endogenous opioids and opioid receptors, and listed the commonly used opioid ligands in research. Several previous reviews have well addressed the history of opioid research, classification of opioid receptors, signal transduction properties of these receptors with regards to G-protein coupling, adenylyl cyclase and cAMP as well as early studies of opioid receptor function (especially on opioid-induced analgesia and tolerance/dependence) [32–43].

In spite of extensive research, controversies still linger. We have attempted to present a comprehensive and objective overview on this topic, though we may not necessarily agree with all the conclusions proposed by the original articles. In this way, we believe, this review shall provide an informative reference for better understanding the opioid system in the body and for further elucidation of the opioid receptor function in a physiological and pharmacological view.

2. ENDOGENOUS AND EXOGENOUS OPIOIDS

Acheson [44] coined the term "opioids" that broadly covered all compounds with morphine-like action and distinct chemical structures ranging widely from alkaloids to peptides. Throughout history, they have been widely used as analgesics to combat pain or induce ecstasy in medical and non-medical situations. Despite the long history of usage, the underlying mechanisms the opioid action are largely unknown. In 1960's, Tsou and Jang [45] performed a pioneering work in understanding the mechanism through the direct microinjection of morphine into the brain to produce analgesia, which provided the impetus for studies on role of opioids in brain function. Unfortunately, their interesting work was

never formally published in any international journal owing to the prevailing political unrest at that time in China. By the early 1970's, Pert and Yaksh [46, 47] published their data delineating sites of morphine-induced analgesia in the primate brain. Furthermore, Liebeskind and colleagues observed that brain stimulation in certain areas, particularly the periaqueductal gray, caused pronounced analgesia [48] that was blocked by the opioid antagonist naloxone [49]. This observation strongly suggested the existence of endogenous opioids in the brain. Shortly thereafter, two groups independently reported that brain extracts mimicked the ability of morphine to inhibit electrically induced contractions of the mouse vas deferens, which was blocked by naloxone [50, 51]. These discoveries gave rise to the identification and isolation of two enkephalin pentapeptides, namely [Met^5]enkephalin (H-Tyr-Gly-GlyPhe-Met-OH) and [Leu^5]enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) [50], followed closely by the identification of β-endorphin [52, 53] and dynorphin [54] in brain tissue. In 1995, an endogenous agonist for the opioid receptor-like orphan receptor (ORL) was isolated concurrently by two independent groups, which was termed ORL-1 by Mollereau *et al.* [55] and Meunier *et al.* [56], and orphanin FQ by Reinscheid *et al.* [57]. It is commonly referred to as nociceptin. Two years later, endomorphin-1 and -2, endogenous MOR agonists with very high affinity and selectivity were synthesized and subsequently isolated by Zadina [58] in bovine and human brain extracts [58, 59]. So far at least ten endogenous opioids have been identified in the brain, which belong to distinct families of compounds (Table 1).

Endogenous opioids can originate from the same or different precursor proteins. All mammalian opioid peptides are derived from three precursors, i.e., pro-opiomelanocortin (POMC), pro-enkephalin (PENK) and pro-dynorphin (PDYN), which are translated from separate genes [60]. Although the sequence of [Met^5]enkephalin is contained in the sequence of POMC [61], POMC is not the source of [Met^5]enkephalin peptide. Instead, [Met^5]enkephalin is encoded by the preproenkephalin gene. A 31-amino-acid portion of β-lipotropin was isolated and named as β-endorphin [52]. It possessed an affinity for μ-opioid receptors. Subsequently, a series of polypeptides containing the N-terminal [Leu^5]enkephalin sequence were isolated and designated as another opioid group called dynorphins [54]. Cloning of the peptide precursors demonstrated that one precursor contained both [Met^5]enkephalin and [Leu^5]enkephalin transcripts, a second precursor existed for the dynorphin peptides, and a third for β-endorphin and other pro-opiomelanocortin peptides (adrenocorticotrophin and melanoctye-stimulating hormone) [62]. Many C-terminal extended enkephalins were also identified and demonstrated distinct spectra of bioactivities [63]. Till date, however, the precursor for endomorphins remains to be discovered.

Upon identification of the unique properties of opioids, especially their effectiveness in producing analgesia, many exogenous opioids were synthetically developed to enhance their properties for an eventual application in clinical research. However, among the vast plethora of compounds recognized in the literature during the last several decades, morphine still remains the most popularly used exogenous opioid for pain relief, since it is the quintessential MOR agonist and exhibits higher affinity for MOR and than for either DOR or KOR [64, 65]. While many exogenous opioids (Table 2) with similar pharmacological properties as morphine have been used both clinically and in laboratory, they however differ widely with respect to their efficacies, potency, side-effects, clearance, and passage through the blood-brain barrier [66, 67].

3. OPIOID RECEPTORS

The action of opioid compounds is mediated through activation of specific opioid receptors. Three major opioid receptor families, the μ-, κ-, and δ-opioid receptors, were cloned in

early 1990s [32, 34] and a fourth member of the opioid receptor family, nociceptin or orphanin FQ receptor (NOP) or the opioid receptor-like orphan receptor (ORL), was added to the list in 1994 [55].

Opioid receptors exist not only in the nervous system [68–73], but also in peripheral organs, such as heart, lungs, liver, gastrointestinal and reproductive tracts [74, 75]. However, the expression and distribution of these receptors vary significantly among different organs as well as among different animal species [36].

3.1. Major Opioid Receptor Family

Opioid receptors have high sequence homology and belong to the large family of seven-transmembrane G protein-coupled receptors. Because of the homologies between ORL/NOP and other opioid receptors, functional studies have also focused primarily on pain and analgesia [76–82]. Other functions of ORL/NOP receptors include dynamic homeostatic mechanisms, since they are distributed throughout the body [39, 83, 84].

In general, endogenous opioid peptides are not highly selective or specific to one particular type of opioid recaptors, except the endomorphins [58]. This is due to three major factors: (1) all peptide ligands contain the N-terminal residue Tyr (except Phe in the case of nociceptin), comparable to a functional hydroxyl group in the morphinans, which is a requirement for interaction within the ligand-bind domain of opioid receptors; (2) MOR, DOR and KOR have many common structural similarities in their primary structures, in addition to function and intracellular signaling mechanisms; and (3) the formation of homomeric and heteromeric complexes between opioid receptors and non-opioid receptors modify their response to a given opioid ligand [32, 34–36, 38, 39, 85]. In other words, the outcome of opioid action could be the result of an interaction with different opioid receptor complexes. On the other hand, synthetic opioid peptides and alkaloids have a relatively high selectivity to MOR, DOR, and KOR and have been widely used to define the pharmacological properties of opioid receptors.

Studies suggest that the physical interactions between opioid receptors play a major role in defining their resultant pharmacological and physiologically behaviors [86–89]. For example, δ-agonists were shown to enhance the analgesic potency and efficacy of μ-agonists, and δ-antagonists prevented or reduced the development of tolerance and physical dependence by μ-agonists [38]. Another example is LY255582, an opioid antagonist, which produced effects on feeding and body weight gain through a heteromeric combination of MOR, DOR and/or KOR [90]. Therefore, new ligands with dual μ-/δ-agonism or μ-/δ-antagonism, or mixed μ-agonism/δ-antagonism or vice versa are emerging as promising unique approaches to the development of analgesic drugs [38, 91, 92].

3.2. Opioid Receptor Subtypes

The development of novel opioid analogs that elicited different biological effects gave way to the detection of pharmacological subtypes of MOR, DOR and KOR, although the molecular basis of these subtypes has not yet been established [93–96] and, to date, only one μ-opioid receptor gene (Oprm) has been isolated. Of the three opioid receptor families, MOR subtypes have been most extensively studied due to their role in mediating the actions of morphine and other clinically relevant analgesic agents as well as drugs of abuse. These observations gave rise to the concept of multiple μ-receptors along with the cross-tolerance to different μ-opioids [97]. Genetic and physiological variations influence the sensitivity of MOR towards different μ-opioid ligands and difference in their potency across various species and animal strains [34], which led to the proposal that receptors could be classified as MOR1, MOR2, and MOR3. Similarly, KOR multiplicity is also a complex issue under

debate. Clark *et al.* [98] presented evidence from binding studies in guinea-pig cerebellum that suggested the existence of four KOR subtypes, KOR1a, KOR1b, KOR2 and KOR3, based on pharmacological studies [99, 100]. In addition, DOR has been demonstrated to exist in at least two subtypes, DOR1 and DOR2, according to their pharmacological attributes [94, 96, 101–104]. Rothman first proposed the existence of DOR subtypes [105, 106] in which a δ-site associated with the μ- δ-opioid receptor complex was termed the δcx binding site, while non-associated or non-complexed δ-site as the δncx site, which corresponded to DOR1 [107], however, this complex terminology is not used in the opioid literature. Although many studies have been conducted on opioid receptor subtypes, an important question that hovers over the issue of various subtypes is the lack of a specific cDNA corresponding to a receptor subtype, e.g., only one receptor gene has been isolated for each opioid receptor in spite of the existence of the multiple subtypes [108–110]. DOR has been cloned in several mammalian species including mouse [111–113], rat [114, 115] and humans [116], yet no cDNA corresponding to DOR1 and DOR2 has been found.

The emerging notion on the occurrence of pharmacological subtypes of opioid receptors as a result of receptor heteromerization that leads to new pharmacological properties is largely being accepted. The properties of these heteromerized receptors are often found to be similar to those of the previously described subtypes [85, 110]. The subtypes of opioid receptors may also be considered as splice variants of the same gene, since six new splice variants from the human μ-opioid receptor gene have been identified and characterized yielding a total of 10 human splice variants of MOR1 that differed in both potency and efficacy [117]; nonetheless, all variants were equally selective for μ-opioid ligands, thereby, confirming their classification as μ-opioid receptors [118].

3.3. Commonly Employed Opioid Agonist and Antagonist Ligands

Some common opioid agonist and antagonist ligands used clinically or in research are listed in Tables 2 and 3 for reference, but the list is by no means complete as it would be quite extensive and beyond the scope of this overview. It should be noted, however, that the selectivity mentioned here is based on the predominant spectrum of activity, concentration used for *in vivo* studies, the area of brain in which they are infused or injected, for example, naloxone and naltrexone are relatively non-selective opioid receptor antagonists similar to diprenorphine. Many integrated factors comprise receptor selectivity.

4. ADVANCES IN RESEARCH ON OPIOID RECEPTOR FUNCTIONS

Opioid peptide receptors in the CNS represent the most extensive and diverse peptidergic transmission system and are widely involved in various pleiotropic functions. They are essential for various physiological functions, including pain modulation, locomotion, mood, diuresis, thermoregulation and stress, along with regulatory functions in, respire-tory, gastrointestinal and cardiovascular systems. On the other hand, abuse of opioid compounds leads to addiction, which greatly affects brain function and body homeostasis.

4.1. Ionic Homeostasis

Recent studies show that opioid receptors are involved in the regulation of ionic homeostasis. Under normal conditions, there is a steep electrochemical gradient across the plasma membrane with the extracellular fluid rich in Na^+ with concentrations up to 150 mM and relatively poor in K^+ (ca. 3 mM), and a reverse intracellular environment with up to 150 mM K^+ and 4–12 mM Na^+. Maintenance of ionic homeostasis is vital for normal functioning of the neurons. Opioids have been shown to be involved with the regulation of ionic homeostasis under both normoxic and hypoxic/ischemic conditions. All opioid agonists either induce an elevation of intracellular Ca^{2+} or lead to an inhibition of Ca^{2+} entry

under normal conditions. However, the overall predominant effect of opioids on Ca^{2+} homeostasis is inhibitory [120–126], which seems to be consistent with classical recognition of the endogenous opioid system as an inhibitory regulator in the brain. In contrast, some studies show that opioid receptor activation activates Ca^{2+} channels. For example, the MOR agonist DAMGO (10 nM) produces a small yet consistent facilitation of Ca^{2+} current through P-type calcium channels via G-protein-independent mechanism in the rat Purkinje neurons [127]. The opioid-induced elevation of intracellular Ca^{2+} could explain the excitatory effect of opioids on some presynaptic neurons and consequent regulation of neuro-transmitter release [128, 129]. Recently, we assessed the effect of DOR and MOR activation/inhibition on K^+ and Na^+ homeostasis in mouse cortical slices by directly measuring the extracellular K^+ and Na^+ concentrations with ion-sensitive microelectrode [130–135]. The results revealed that 20 mins of δ-agonists DADLE or UFP-512 [136], or DAGO (δ-agonist) and naltrindole (δ-antagonist) perfusion under normoxia, produced no obvious change in the extracellular K^+ and Na^+ concentrations in cortical slices, suggesting little effect of DOR and MOR on K^+ and Na^+ homeostasis under normoxic conditions [130–135]. However, activation of DOR, but not MOR, greatly attenuates hypoxia/ischemia-induced increase in extracellular K^+ and decrease in extracellular Na^+, which suggests that opioids are one of the important factors in the regulation of ionic homeostasis under environmental stress.

4.2. Cell Proliferation

Opioid receptors affect cell proliferation. Malendowicz *et al.* [137] observed that μ- and δ-receptor activation inhibits the growth of immature adrenals, stimulates adrenal regeneration, and does not affect proliferation of cultured adrenocortical cells. However, Narita *et al.* [138] found that the stimulation of δ-opioid receptors by the δ-opioid receptor agonist SNC80 promoted neural differentiation from multipotent neural stem cells obtained from embryonic C3H mouse forebrains. In contrast, either a selective μ-opioid receptor agonist DAMGO or a specific κ-opioid receptor agonist U-50488 hydrochloride (U50, 488H) had such effect. These findings have mooted the concept that δ-opioid receptors play a crucial role in neurogenesis and neuroprotection. Some recent studies have shown that the OGF-OGFr (opioid growth factor-opioid growth factor receptor) system is a native biological regulator of cell proliferation in some cancers including ovarian cancer, hepatocellular cancer, and so on [139, 140]. More studies are, however, needed for the validation of this interesting observation and its underlying mechanisms.

4.3. Neuroprotection

One of the most exciting findings of the past decade is the opioid-receptor, especially DOR, mediated neuroprotection against hypoxic/ischemic injury.

Historically, there have been major controversies over the role of opioids in the neuronal responses to hypoxic/ischemic insults. Some investigators [6,7, 141–143] have demonstrated that opioid receptor activation, by opioid agonists, protects brain from ischemia and extended the animal survival time during severe hypoxia, while others [1–4, 144, 145] showed opioid receptor inhibition by intravenous injection of high dose naloxone that interacts with DOR, MOR and KOR thereby protecting the brain from ischemia-induced injury.

Our serial studies since 1997 have demonstrated that DOR is neuroprotective against hypoxic/excitotoxic stress in [8–15, 43, 130, 146, the brain, especially cortical neurons 147] and clarified the historical controversies on the role of opioids and their receptors in hypoxic/ischemic neuronal injury [16, 42]. Furthermore, we have found that the mechanisms for the DOR neuroprotection involves the stabilization of ionic homeostasis [43, 130–134],

increase in intracellular transduction of pro-survival signals [13, 132] and attenuation of oxidative injury [15] as well as regulation of DOR expression [12, 13, 135].

We applied glutamate to cultured cortical neurons to mimic neuro-excitotoxicity since it is a key mediator of ischemic/hypoxic injury. The addition of glutamate induced neuronal injury and cell death in a dose-dependent manner with a major difference between the immature and mature neurons. Activation of DOR with 10 μM DADLE reduced glutamate-induced injury by almost half and naltrindole (a DOR antagonist, 10 μM) completely blocked this protective effect. In sharp contrast, administration of MOR and KOR agonists (DAMGO and U50, 488H respectively, 5–10 μM) did not induce appreciable neuroprotection, and MOR or KOR antagonists had no significant effect on glutamate-induced injury. These data demonstrate that activation of DOR, but not MOR and KOR, protected neocortical neurons from exerts neuroprotection against glutamate excitotoxicity [8, 9]. DOR neuroprotection against hypoxic stress, ischemic insult or excitotoxic injury was shown in our serial studies [8–15, 43, 130, 146, 147] and has been well demonstrated in many other laboratories [18–31, 138].

4.4. Hibernation

Mammalian hibernation is a distinct energy-conserving state and a potential protective strategy for the peripheral and central nervous system, which is associated with depletion of energy stores, intracellular acidosis and hypoxia similar to the changes that occur during ischemia. Many physiological features in hibernation including analgesia and respiratory depression can be triggered by DOR activation [21]. Circulating levels of opioid peptides in hibernating animals increased dramatically, and are considered a "hibernation induction trigger" [148–151]. In the brain, the hypothalamic and septal concentrations of [Met^5]enkephalin were found to be significantly higher in the hibernating state than that in the non-hibernating state [152], while the levels in the pons and medulla showed little change [153]. Endogenous opioid activity is seasonally affected in the lateral septal region of the brain of hibernating animals with an increase in endogenous opioid activity during hibernation, which is probably involved in regulation of hibernation cycle [154]. Accompanying the increase in the concentration of opioid peptides, opioid receptor (MOR, DOR and KOR) levels in the brain of hibernating animals decreased, which could be an adaptation to survive under thermal stress [149]. Administration of opioid antagonists effectively reversed hibernation and aroused the animals from their stupor [150, 155]. These findings clearly suggest that endogenous opioids, especially DOR, play a significant role in hibernation.

4.5. Pain Modulation

A majority of studies on opioids are associated with analgesia [156–158]. Many acute insults result in the release of endogenous opioids and increase in opioid levels in blood, which counteracts the noxious stimulus and pain, i.e., stress-induced analgesia by opioids. Circulating levels of β-endorphin are significantly elevated following muscle injury, fixed-pressure hemorrhagic shock and lipopolysaccharide (LPS) administration in animal models, which serves as an index of endogenous opioid system activation [159]. Similar stress-induced elevations in the production and release of endogenous opioids have been demonstrated in clinical studies, oral surgery [160] as well as gynaecological and abdominal surgeries [161, 162] that resulted in increased β-endorphin levels in patients. Stress-induced analgesia can be partially reversed by the broad-spectrum opioid antagonist naloxone [163], further emphasizing the involvement of endogenous opioids in this process. It is generally accepted that endogenous opioids tonically regulate nociceptive information and that all naturally occurring exogenous opioid agonists produce analgesia. Today, opiate drugs are widely used in the treatment of post-operative pain.

Opioid knockout receptor models provide a powerful tool for studying analgesia and other physiological effects attributed to the opioid system [156]. Strong evidence shows that MOR plays a central role in analgesia. Mice lacking MOR have increased sensitivity to heat, suggesting the existence of a MOR-mediated tone in thermal nociception [156] and a reduction in stress-induced analgesia [164]. In contrast, DOR- and KOR-deficient mice did not exhibit any alteration in heat perception. Many of the currently available clinical opioid analgesics exert their effects primarily through MOR [38]. After knocking out MOR, the analgesic effects of δ-agonists are either unchanged or diminished [165], while MOR agonists fail to exhibit analgesia [166, 167]. However, DOR agonists can enhance the analgesic potency and efficacy of MOR agonists and the antagonists can prevent or reduce the development of tolerance and physical dependence to MOR agonists [38]. All these observations indicate a vital role of MOR in pain modulation.

Immunohistochemical and ultrastructural analysis revealed that DOR exists in peripheral nerves in the molar dental pulp [168], and that bradykinin, an inflammatory mediator, causes a reaction in trigeminal ganglia sensory neurons, which results in a rapid increase in significant accumulation of DOR in the plasma membrane thus contributing to peripheral opioid analgesia [168, 169]. It was observed in rodent models that DOR1 and DOR2 antagonists respecttively blocked the supraspinal anti-nociceptive activation by DOR agonists DPDPE and deltorphin [101–104]. In DOR knockout animals, DOR agonist-induced analgesia was either abolished, reduced, or unchanged, depending on the nociceptive assay and route of administration [170]. The recorded analgesia could be a result of the cross-reactivity of DOR agonists at MOR *in vivo* [165]. Contrary to the pre-demonstrated that, under vailing view, Scherrer *et al.* [171] resting conditions, DOR is trafficked to the cell surface independent of substance P and internalized following activation by DOR agonists. There is also evidence showing that the segregated DOR and MOR distribution is paralleled by a remarkably selective functional contribution of the two receptors to the control of mechanical and thermal pain, respectively.

KOR also plays a role in pain modulation. Though KOR-deficient did not exhibit any alteration in the perception of thermal or mechanical pain, KOR mutant mice had an enhanced pain response to the peritoneal acetic acid injection [165]. Dynorphin-deficient mice also displayed a mild increase in pain induced by inflammation [172]. Compared to MOR and DOR, KOR chiefly mediates analgesia to visceral pain [173].

In mechanistic investigations, serial studies from Caron and Lefkowitz laboratories using β-arrestin knockout mice show that β-arrestins regulate opioid receptor-mediated signal transduction and hence play an important role in opioid-induced analgesia and tolerance/dependence [174–177]. Pei and Ma [178–181] have also confirmed these findings.

4.6. Drug Abuse/Addiction

Excessive use of opioids leads to opioid addiction. Drug abuse and addiction are now considered as neurological pathology due to their effects on brain function. Neurological impairments observed in addiction reflect drug-induced neuronal dysfunction and neurotoxicity. The drugs of abuse directly or indirectly affect various neurotransmitter systems, particularly dopaminergic and glutamatergic neurons [182, 183]. The treatment of drug abuse and addiction is the most expensive of all the neuropsychiatric disorders associated with personality disorders and a diminished quality of life [182, 184, 185]. Behavioral patterns of addiction include compulsive drug-seeking, persistent abuse of substances despite the often irreparable social consequences and deterioration of physical health, and the high probability of relapse even after prolonged drug-free periods [186].

The mechanisms underlying neurological disorder caused by opioid addiction are not clear yet. Neuronal basis of positive reinforcement relies on activation of dopaminergic neurons resulting in an increased dopamine release in the mesolimbic brain structures. Certain aspects of opioid dependence and withdrawal syndrome are also related to noradrenergic and serotonergic systems, as well as to both excitatory and inhibitory amino acid and peptidergic systems. An important role in neurochemical mechanisms of opioid reward, dependence and vulnerability to addiction has been ascribed to the activation of endogenous opioid peptides, particularly those acting via the MOR and KOR.

Opioid abuse leads to opioid tolerance in the nervous system. Receptor tolerance and adaptation involve complex mechanisms of receptor regulation, including desensitization and internalization [187]. Several important processes have been identified including upregulation of cAMP/PKA and cAMP response element-binding signaling and perhaps MAPK cascades in opioid sensitive neurons, which might not only influence tolerance and withdrawal, but also synaptic plasticity during the cycles of intoxication and withdrawal [188]. Intracellular molecules of signal transmission are also involved in opioid tolerance and dependence, including G proteins, cyclic AMP, MAP kinases, and some transcription factors. The latter link in this chain of reactions modifies the expression of target genes, and it may be responsible for the long-lasting neural plasticity induced by opioids [188, 189]. In general, drug abuse induces adaptive changes in opioid receptors that occur following acute (e.g., desensitization and/or internalization) and chronic (e.g., adaptive tolerance and down-regulation) opioid administration [190–193]. Future work on prescribed drugs of abuse will include identification of clinical practices that minimize the risks of addiction, the development of guidelines for early detection and management of addiction, and the development of clinically effective agents that minimize the risks for abuse [194].

In addition, opioid abuse may predispose an individual to opportunistic infections, such as hepatitis, bacterial pneumonia, tuberculosis, abscesses, CNS infections, endocarditis and even AIDS [195–197]. One of the explanations for this phenomenon is that opioids suppress immune function (also refer to *4.9* below) including antimicrobial resistance, antibody production, monocyte-mediated phagocytosis, and neutrophil and monocyte chemotaxis [196].

4.7. Emotional Response

The role of the opioid system in controlling pain, reward and addiction is well established, but its role in regulating other emotional responses is poorly documented in pharmacology [198]. Recent studies have shown that endogenous opioid systems are associated with the regulation of emotional responses. In particular, it has been reported that δ-opioid receptors act as natural inhibitors of stress and anxiety [199]. DOR agonists have been shown to produce antidepressant and anxiolytic effects in rodent models. In addition, DOR agonists have been shown to increase expression of brain-derived neurotrophic factor (BDNF) mRNA, an effect of some antidepressants, which may be important for the clinical efficacy of antidepressant drugs [200, 201]. Zhang *et al.* [202] reported that centrally administered nor-BNI, like most clinically used antidepressants, upregulated BDNF mRNA expression in the rat hippocampus. These findings further imply that central KOR mediates antidepressant-like effects by BDNF gene upregulation. Some data demonstrate dynamic changes in MOR neurotransmission in response to an experimentally induced negative affective state [203].

In addition, stress contributes to opioid-induced neuronal activation. Mice exposed to stress and morphine showed region-specific increases in KOR [204, 205].

4.8. Epileptic Seizures

The role of opioid system in epileptogenesis and epileptic seizure is still controversial. Both anticonvulsant and procon-vulsant effects of the three major opioid systems (δ-, μ-, and κ-) as well as nociceptin opioid peptide have been proposed in the literature [206–219]. For example, Rubaj *et al.* [220] showed that MOR and KOR are involved in hypoxic preconditioning against seizures in the brain. They observed that an episode of normobar hypoxia reduced the susceptibility to convulsions induced by pentylenetetrazol, which could be mimicked by MOR or KOR agonists.

In some experimental models of epilepsy, endogenous opioid (e.g. enkephalin and dynorphin) levels are greatly increased in the brain during epileptic seizures [209, 219, 221], and opioid receptors are up-regulated following spontaneous epileptic seizure [222]. It is not clear if this represents a compensatory mechanism against epilepsy because multiple factors determine the anticonvulsant or proconvulsant role of opioids in the brain. For example, morphine depresses electrographic seizure activity under a low concentration, while enhances seizure activity under high concentrations with an apparent dose-dependent manner [217, 223]. SCN80, a non-peptide DOR agonist, both in low or high dose, produced convulsions in the rat, but had little effects in rhesus monkeys [215, 224, 225]. Retarding the rate of administration of SCN80 brought a reduction in its convulsive effect in the rat [226]. Also, the action of opioids depends on the site of action. Endogenous opioids generally play an inhibitory role in modulation of neuronal excitability. However, if this inhibition occurs in inhibitory interneurons, the opioids facilitate, rather than suppressing seizures via post-synaptic de-inhibition. Therefore, increased familiarity with the effects of opioid action on epilepsy is necessary.

Data from our recent work shows that DOR inhibits sodium channels and thus suppresses epileptic activity in the cortical regions. Sodium channel mutation has been casually linked to human epilepsy [227, 228], and an up-regulation of Na^+ channels has demonstrated to cause epileptic hyper-excitability and seizures [229, 230]. Interestingly, we observed that in the mutant brain exhibiting spontaneous epilepsy, Na^+ channels are up-regulated [230], while DOR is down-regulated [231], implicating DOR contribution to the pathophysiology of epilepsy associated with genetic abnormality. In addition, our studies on Na^+ channels and DOR co-expression in Xenopus oocytes demonstrated that DOR activation inhibits Na^+ channel function by decreasing the amplitude/velocity of sodium currents and increasing their threshold for activation [135]. Since Na^+ channels play a major role in neuronal excitability including epileptic hyper-excitability and most of anti-epileptic drugs are actually inhibitors of Na^+ channels [232, 233], the phenomenon we observed can provide novel clues for better solutions of epileptic seizures.

4.9. Immune Function

Opioids may behave like cytokines to modulate the immune response through an interaction with their receptors in central and peripheral neurohumoral systems [234–237]. In general, opioids mediate immunosuppression although their actions are complicated, often indirect and not well understood. Nonetheless, acute and chronic opioid administration is known to have inhibitory effects on humoral and cellular immune responses including antibody production, natural killer cell activity, cytokine expression, and phagocytic activity [235, 236, 238–240]. *In vivo* and *in vitro* studies indicated that opioid receptor stimulation exerts suppression of multiple components of the immune defense response including natural killer (NK) cell activity [241], neutrophil complement and immunoglobulin receptor expression [242], chemokine-induced chemotaxis [243] and phagocytosis [244]. Bai *et al.* [245] found that after intracerebroventricular injection of β-endorphin in rats, the mitogen-induced spleen lymphocyte DNA synthesis, hemolysin formation and IgG production were reduced

significantly, suggesting a role of the central opioid system in immune regulation. Pretreatment with U50 488 - a κ-opioid ligand, significantly reduced lipopolysaccharide (LPS)-stimulated interleukin-6 (IL-6) production. This effect was mediated by the KOR through inhibition by KOR antagonist-nor-binaltorphimine (nor-BNI) [246]. Treatment of normal human astrocytes with morphine leads to significant down-regulation of gene expression for β-chemokines, MCP-1, and MIP-1β, while reciprocal up-regulation of the expression of their specific receptors, CCR2b, CCR3, and CCR5, respectively, and reversed by naloxone [247]. In addition, acute administration of morphine significantly decreased NK cell cytotoxicity and interferon-gamma mRNA levels, and increased the mortality rate of mice infected with herpes simplex virus 1 [240]. DOR agonist, DPDPE, triggers monocyte adhesion [248]. Ordaz-Sanchez *et al.* [196] observed that SNC80, another DOR agonist, significantly stimulated rat thymic and human leukocyte chemotaxis. The effects of SNC80 on the chemotaxis of rat and human leukocytes were antagonized by naloxone, indicating that the modulation of chemotaxis via opioid receptors. It has been suggested that activation of the KOR induces an anti-inflammatory response through the down-regulation of cytokine, chemokine and chemokine receptor expression, while activation of MOR favors a pro-inflammatory response [249]. Investigation into the ORL1/nociceptin system demonstrated a down-regulation of immune function secondary to the activation of this receptor [39, 249].

In summary, opioid modulation of the immune response in animals is mediated through the direct interaction with opioid receptors expressed by immune cells. The influence of the opioids on the immune response *in vivo* is likely to be a result of involvement of both the central nervous system and the hypothalamic-pituitary-adrenal axis [250]. Sharp *et al.* [251] demonstrated that the β-endorphin and dynorphin stimulate the superoxide production by neutrophils and peritoneal macrophages. Inhibition of superoxide production by morphine is likely to be mediated via soluble factors, such as growth factor β, produced by the lymphoid cells present in the peripheral blood [252].

Pharmacological evidence has demonstrated the presence of MOR, KOR and DOR, as well as non-classical opioid-like receptors, in cells of the immune system. Studies have shown that the phagocytic activity of non-elicited macrophages is inhibited by *in vitro* administration with μ-, κ-, or δ-opioid agonists [253]. An antagonist blocks the inhibitory activity of each agonist with the appropriate opioid receptor selectivity.

TNF-α and IL-6 are the two of the major cytokines secreted by activated macrophages. One study [254] showed that morphine differentially modulates LPS induced expression of IL-6 and TNF-α, and Greeneltch *et al.* [255] found that naltrexone was capable of preventing LPS-induced septic shock mortality by indirect inhibition of TNF-α production *in vivo*. Although Alicea *et al.* [256] reported that macrophage function is significantly modulated following activation of the KOR, morphine and the endogenous opioids (β-endorphin, [Met^5]enkephalin, and dynorphin) also induce chemotaxis of human monocytes and neutrophils [243, 257–259]. Morphine modulation of several immune functions, including macrophage phagocytosis and macrophage secretion of TNF-α, was not observed in the MOR knock-out animals suggesting that these functions are mediated by the classical MOR receptor [254].

4.10. Feeding

Opioid receptors also participate in the regulation of feeding. Opioid agonists and antagonists have corresponding stimulatory and inhibitory effects on feeding. In addition to studies aimed at identifying the relevant receptor subtypes and sites of action in brain, there has been an increasing interest in the role of opioids on diet/taste preferences, food reward, and the overlap of food reward with others types of reward. Some data suggest a role for opioids in the control of appetite for specific macronutrients, but there is also evidence for

their role in the stimulation of intake based on already-existing diet or taste preferences and in controlling intake motivated by hedonics rather than by energy needs [260].

Agonist stimulation of opioid receptors increases feeding in rodents, while opioid antagonists inhibit food intake and weight gain in ob/ob mice [261]. The pan-opioid antagonist, LY255582, produces a sustained reduction in food intake and body weight in rodent models of obesity. The study on mouse indicated that LY2555823 produces its effects on feeding and body weight gain likely through a combination of μ-, δ- and κ-receptor activity [90]. The κ-antagonist, nor-binaltorphimine, significantly increased food intake, while naltrexone (general antagonist) and naloxonazine (μ-antagonist) both reduced feeding [262]. Research suggested that syndyphalin-33 (SD33) - a μ-opioid receptor ligand, increases food intake in sheep after intravenous injection, and its effects are mediated via opioid receptors [263].

4.11. Obesity

Opioid receptors as obesity-related factor affect body weight and obesity. Studies showed that stimulation of μ-opioid receptors preferentially increases the intake of a high fat diet. The increased levels of hypothalamic μ-opioid receptors in Osborne-Mendel rats could contribute to their preference for a high fat diet and increased susceptibility to obesity [264, 265]. The μ-opioid receptor signaling in the nucleus accumbens core and shell is necessary for palatable diet-induced hyperphagia and obesity to fully develop in rats [266]. Czyzyk *et al.* [267] fed KOR-knockout (KO) and wild-type (WT) mice with high-energy diet (HED) for 16 wk. They found that the KO mice had 28% lower body weight and 45% lower fat mass when compared to WT mice fed an HED. Furthermore, KOR deficiency led to an attenuation of triglyceride synthesis in the liver. This study provides the evidence that KOR plays an essential physiological role in the control of hepatic lipid metabolism, and KOR activation is a permissive signal toward fat storage. Marczak *et al.* [261] administered H-Dmt-Tic-Lys-NH-CH_2-Ph (MZ-2), a potent μ-/δ-opioid receptor antagonist, in mice and found that MZ-2 exhibited the following: (1) reduction in weight gain in sedentary obese mice that persisted beyond the treatment period without any significant effect on leaner mice; (2) stimulation of voluntary running behavior on exercise wheels of both groups of mice; (3) decrease in fat content, enhanced bone mineral density (BMD), and decreased serum insulin and glucose levels in obese mice; and (4) MZ-2 (30 μM) increased BMD in human osteoblast cells (MG-63) comparable to naltrexone, while morphine inhibited mineral nodule formation. Thus, MZ-2 offers potential for application in the clinical management of obesity, insulin and glucose levels, and osteoporosis.

4.12. Respiratory Control

Opioids affect ventilation, leading to respiratory depression in both animals and humans [268, 269]. This effect occurs in part due to a direct action of opioids on respiratory-generating structures in the brain [269]. It has been known that there are high densities of opioid receptors in the brain areas related to respiration [270, 271] and local application of opioid agonists to these areas depressed the activity of respiratory related neurons [271, 272]. Opioid receptors show a heterogeneous variation in the depression of respiration by opioids, as MOR activation shows a marked decrease in respiratory frequency and complete apnea, less pronounced respiratory depressant effect by DOR, and the absence of an effect on respiration upon KOR activation [271]. Also, there appears to be "crosstalk" among opioid receptors regarding their depression on respiratory response. For example, it has been shown that respiratory depression produced by DOR agonists is suppressed, while the respiratory response to the activation of KOR is maintained in MOR knockout mice [273]. The phenomenon could be a result of μ-/δ-receptor heteromeric interactions, which exhibit regional selectivity across different areas of the brain [274].

Owing to an extensive clinical use of opioid drugs for pain relief (e.g., cancer-induced or post-operative), the accompanying undesirable side-effect of opioid-induced respiratory depression has become an important concern. Though associated mortality is low, respiratory depression poses a significant clinical problem for patients treated with opioids, especially in the post-operative period [269]. Opioid drugs depress the rate and depth of breathing, blunt respiratory responsiveness to CO_2 and hypoxia, increase upper airway resistance and reduce pulmonary compliance. Opiate respiratory disturbances are mainly due to the agonist activetion of μ- and δ-receptor subtypes and involves specific types of respiratory-related neurons in the ventrolateral medulla and the dorsolateral pons [275, 276].

Under certain conditions, this adverse effect may be lethal. For instance, severe stress (hemorrhagic shock, trauma, bacterial infection) resulting in a massive release of endogenous opioids, or an overdose of opioid analogues (drug abuse, addiction, and uncontrolled use for pain relief) can cause a severe respiratory depression, hypotension, and even death.

4.13. Cardiovascular Regulation

Opioid peptides, including β-endorphin [277], enkephalins [278–281] and dynorphins/ dynorphin-like peptides [280, 282], are present in the heart. Myocardial cells are sites of opioid peptide synthesis, storage and release [283]. Myocardial opioid peptide levels are elevated during episodes of stress, such as ischemia [150, 284]. In addition to heart myocardial cells, δ- and κ-opioid peptides have been shown to exist in sympathetic never fibers and ganglion cells [279, 285]. Short-term cholestasis is associated with resistance against ischemia/reperfusion-induced arrhythmia, which depends on availability of endogenous opioids [286]. Both the KOR agonists, ethylketocyclazocine and enadoline, elicited an increase heart rate with a little effect on blood pressure through a combined action on central and peripheral receptors in conscious squirrel monkeys that could be blocked by the opioid antagonist naltrexone. This effect appeared to be specific for KOR, since the MOR agonist morphine did not mimic the effects of the KOR agonists [287]. In addition, both DOR [172, 288] and KOR [172, 289] have been shown to mediate cardioprotection by preconditioning with myocardial ischemia and metabolic inhibition, one of the consequences of ischemia.

Animal experiments have demonstrated that the opioid system can modulate hemodynamic and cardiovascular activity. In an animal model of hemorrhagic shock, opioid receptor antagonists ameliorated hemodynamic instability [290, 291]. Microinjection of DOR antagonist naltrindole into the ventrolateral periaqueductal gray, a region importantly involved in opioid analgesia, inhibited hemorrhagic hypotension significantly in both conscious and anesthetized rats, while MOR and KOR antagonists were ineffective [292]. However, it is reported that after hemorrhage and during the re-compensatory period, stimulation of δ_2-opioid receptors led to improved mean arterial pressure in conscious, freely moving male rats, and this recovery involved a change in baroreflex sensitivity [293]. Similar effects of enhanced mean arterial blood pressure were also observed in shock patients undergoing treatment with opioid antagonists [294].

MOR has been shown to be involved in the regulation of cardiovascular function. For example, cyclo[*N*ε,*N*β-carbonyl-D-Lys^2,Dap^5]enkephalinamide (cUENK6), a MOR agonist, was found to stimulate excretion of urine, sodium, potassium and cGMP, suppressed the stress-induced elevation in blood pressures and heart rate [295]. Intrathecal injection of endomorphins, elicited a decrease in systemic arterial pressure and heart rate in a dose-dependent manner [296]. As naloxone significantly antagonized the effects of vaso-depression and bradycardia, it is possible that they were elicited by the activation of MOR in the rat spinal cord [296]. In contrast, Mao and Wang [297] reported that microinjection of

endomorphin-1 (EM-1) or nociceptin into nucleus tractus solitarii increased blood pressure and heart rate. These contrary results indicate that the cardiovascular effects are likely to be dependent on the site of action of opioid peptides. Opioid receptors, especially DOR and KOR, are also involved in cardio-protection against ischemic injury. Gross *et al.* [298–300] observed that opioid receptors are important in mediating ischemic preconditioning (IPC) in the heart of dogs. DOR stimulation with selective agonists could mimic the cardio-protective effects of IPC [300]. In an animal model of severe hemorrhagic shock, following DADLE treatment improved hemodynamic stability and a prolonged survival was observed [142]. Although some investigators believe that DOR1 plays an important role in the DOR-mediated cardiac protection, others maintain an opposite view. For instance, Lasukova *et al.* [301] showed that DOR agonist, DPDPE, induced antiarrhythmic and cardio-protective effects in the condition of coronary artery occlusion and reperfusion, and concluded that this protection manifested secondary to stimulation of cardiac DOR1. On the other believes that DOR2 plays the vital hand, Maslov *et al.* [302] role in cardio-protection. They reported that a pretreatment with peripheral and DOR2 antagonists completely abolished the cardioprotective effects of deltorphin II, while DOR1 antagonist 7-benzylidenenaltrexone (BNTX) had no effect. In addition, protein kinase C (PKC) inhibitor chelerythrine and the NO-synthase inhibitor L-NAME (*N*-nitro-L-arginine methyl ester) also reversed the effects of deltorphin II. The nonselective ATP-sensitive K^+ (K_{ATP}) channel inhibitor glibenclamide and the selective mitochondrial K_{ATP} channel inhibitor 5-hydroxydecanoic acid abolished the infarct-sparing effect of deltorphin II. Inhibition of tyrosine kinase (TK) with genistein, the ganglion blocker hexamethonium and the depletion of endogenous catecholamine storage with guanethidine reversed the antiarrhythmic action of deltorphin II but did not change its infarct-sparing action. Based on all these findings, they concluded that the cardioprotective mechanism of deltorphin II is mediated via stimulation of DOR-2. PKC and NOS are involved in both its infarct-sparing and antiarrhythmic effects. Infarct-sparing effect is dependent upon mitochondrial K_{ATP} channel activation while the antiarrhythmic effect is dependent upon TK activation. Endogenous catecholamine depletion reduced antiarrhythmic effects but did not alter the infarct-sparing effect of deltorphin II. This controversy provides additional evidence on the uncertainty over the existence of DOR subtypes. In studies on KOR, Wu *et al.* [289] showed that KOR plays a role in cardioprotection during the delayed phase of protection, while Wang *et al.* [172] demonstrated that KOR activation mediated both the anti-arrhythmic and infarct limiting effects of IPC. Furthermore, Rong *et al.* [303] found that KOR activation attenuated myocardial apoptosis and infarction after ischemia/reperfusion.

4.14. Role in Neurodegenerative and other Diseases

Substantial data shows that opioids inhibit the onset and progression of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis [304]. Several lines of evidence suggest that DOR agonists have a therapeutic effect on Parkinson's disease [305, 306]. Also, activation of KOR effectively ameliorates L-DOPA-induced dyskinesia symptoms in a rat model of Parkinson's disease [307]. Even in the treatment of uremic pruritus, an opioid receptor agonist - nalfurafine, effectively reduced itching, itching intensity, and sleep disturbances [308]. Although the side-effects of opioid receptor activation under certain conditions represents the other side of the coin, For example, a recent study showed that intrathecal injection of morphine after a short interval of aortic occlusion in the rodent model induced transient spastic paraparesis via opioid receptor-coupled effects in the spinal cord [309].

Abundant clinical evidence shows that various movement disorders such as dystonia can develop in patients, with or without pre-existing Parkinson's disease, while emerging from general anesthesia and it can be dramatically reversed by the administration of naloxone.

This indicates an underlying contribution of the fentanyl and morphine that the patient had received to the development of these movement disorders [310]. Zesiewicz *et al.* [311] described a case of 58-year-old man with advanced Parkinson disease who underwent battery replacement for a deep brain stimulator and experienced severe postoperative bradykinesia and rigidity that lasted for 36 hours. The patient was administered fentanyl as an anesthetic during the procedure and as an analgesic periodically during the day after surgery. The severe bradykinesia and rigidity persisted despite reactivation of the deep brain stimulator and immediate reinstitution of Parkinson disease medications, but resolved completely couple of hours after discontinuation of fentanyl. These observations suggest a potential role of MOR in the movement disorders of patients with Parkinson disease. Indeed, a recent study shows that a selective MOR antagonist, ADC5510, can reduce L-DOPA induced dyskinesia in a Parkinson's disease model [312]. It is apparent that MOR has a very different role compared to DOR and KOR in Parkinson's disease.

However, there is lack of in-depth and mechanistic studies for most functions in which they are involved because the majority of the previous, especially early, studies focused on pain modulation and drug addiction via μ-agonist and MOR in the CNS.

5. CONCLUDING REMARKS

The opioid system is considered as one of the most complex and interwoven neurotransmitter systems in the body. The functional effects of opioids are mediated by three distinct opioid receptors with over-lapping distribution and functions in the brain and other major organs. Although opioids are predominantly used for therapeutic pain relief in a clinical setting, other potential roles of the opioid system throughout the body, especially in the brain, are coming into focus with an emergence of new and specific analogues that have brought forth their subtle differences and enabled the investigators to unravel this issue. Since the cloning of the three major opioid receptors, many studies have been performed to elucidate their function and show that besides the involvement of pain modulation, opioid receptors are widely involved in various pleiotropic functions throughout the body, including ionic homeostasis, cell proliferation, emotional response, epileptic seizures, immune function, feeding, obesity, respiratory and cardiovascular control. They also play a critical role in hibernation. One of the most exciting findings in the past decade possibly is neuroprotection and cardioprotection mediated by opioid receptors, especially DOR. The underlying mechanisms involve the stabilization of ionic homeostasis in acute stage of hypoxic/ischemic stress, up-regulation of survival molecules and anti-oxidative capacity, and the down-regulation of apoptosis during the long-term stress of hypoxia/ischemia. These recent findings can have significant clinical implications with regards to the role of opioids, either endogenous or exogenous, in limiting the pathogenesis of brain and heart from hypoxic/ischemic damage.

Many important issues such as the difference between MOR and DOR, despite structural similarity among them, in terms of neuroprotection still remain to be clarified. Morphine protects purkinje cells against cell death under *in vitro* simulated ischemia-reperfusion conditions, which is mediated by DOR, but not MOR [18]. In rat hippocampal slices, however, exposure to morphine aggravates the neurotoxic effects of a subsequent hypoxia/hypoglycemia in a concentration-dependent manner [313, 314]. It seems that opioid receptors have differential roles in neuronal responses to hypoxic/ischemic stress across different brain regions/cells (e.g., cortical *vs.* hippocampal cells). MOR may induce a toxic effect on neurons in some brain regions under hypoxic/ischemic condition. It is still unclear whether this is a reason why some investigators observed neuroprotection induced by opioid blockers such as naltrexone under certain conditions [315]. More intricate and in-depth studies are needed to address many complex issues like this. Although the role of DOR in

Table 3

Some Notable Opioid Antagonists and Predominant Receptor Selectivity

Ligand	Selectivity	Ligand	Selectivity
β-FNA	μ	BNTX	δ_1
Naltrexone	μ	Naltriben	δ_2
CTAP	μ	[*N*-allyl-Dmt[1]]endomorphin-1,2	μ
CTOP	μ	ICI 174,864	δ, δ_{nex}
Cyprodime	μ	DALCE	δ_1, δ_{ex}
Naloxonazine	μ	GNTI	κ
Naloxone	μ	TRK-820	κ
ADL5510	μ	5-AMN	κ
ADC5510	μ	5-MABN	κ
DIPP-$NH_2\psi$	δ	Nor-BNI	κ
ICI-174864	δ	U50488H	κ
Naltrindole	δ	Diprenorphine	μ, δ, κ
N,*N*(Me)$_2$-Dmt-Tic-OH	δ	MZ-2	μ, δ
TIPP	δ	LY255582	μ, δ, κ
UFP-501	δ		