

Opioid Peptides and Receptors

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The Opioid System: A Short History

Discovery of the opioid system stems from the use and abuse of opium in ancient history. Opium, extracted from poppy seeds (*Papaver somniferum*; see [Figure 1\(a\)](#)), has powerful pain-relieving properties and produces euphoria. This substance has been used both medicinally and recreationally for several millennia. Morphine, named after the god Morpheus, is the most active ingredient of opium. The compound was isolated in 1805 and rapidly became the clinical treatment of choice to alleviate severe pain. Heroin was synthesized chemically by morphine diacetylation in the late 1800s and was commercialized as the first nonaddictive opiate to treat cough and asthma. The strong addictive properties of heroin were soon acknowledged, and both heroin and opium were prohibited in 1910. Today morphine remains the best pain-killer in contemporary medicine, despite a wide array of adverse side effects (respiratory depression, constipation, tolerance, and dependence). Heroin is a main illicit drug of abuse, and heroin addiction represents a major public health issue. Because of their extraordinarily potent analgesic and addictive properties, opiates have prompted scientists to seek to understand their mode of action in the brain.

In 1973, three independent teams showed that opiates bind to membrane receptors in the brain, and these receptors were named μ , δ , and κ a few years later. In 1975, two pentapeptides, Met- and Leu-enkephalin were isolated as the first endogenous ligands for these receptors. Many peptides followed, forming the opioid peptide family. Three distinct genes encoding the peptides were isolated in the late 1970s and early 1980s; three other genes encoding the receptors were cloned a decade later. In the mid 1990s the entire endogenous opioid system was characterized at the molecular level ([Figure 1\(b\)](#)).

Molecular Components of the Opioid System

The Peptides

Opioid peptide genes encode large precursor proteins, which are further cleaved into shorter peptides. All opioid peptides share a common N-terminal Tyr-Gly-Gly-Phe signature sequence, which interacts with

opioid receptors. The preproenkephalin gene encodes several copies of the pentapeptide Met-enkephalin and one copy of Leu-enkephalin. This gene also encodes longer versions of Met-enkephalin. Notably, one of them, the opioid peptide bom22 , activates opioid receptors via its N-terminal sequence while activating another receptor family known as sensory neuron-specific (SNS) receptors via its C-terminal part. The preprodynorphin gene also encodes multiple opioid peptides, including dynorphin A, dynorphin B, and neo-endorphin. The preprodynorphin-derived peptides all contain the Leu-enkephalin N-terminal sequence and basic amino acid residues in their C-terminal end. Finally the preproopiomelanocortin gene encodes a single opioid peptide, β -endorphin, among other peptides with nonopioid biological activity. β -endorphin is the longest opioid peptide (31 amino acids) and shows highly potent and long-lasting analgesic properties.

The Receptors

Opioid receptors are membrane receptors with a seven-transmembrane topology that belong to the large G-protein-coupled receptor (GPCR) superfamily. This family comprises several hundred members within the mammalian genome. μ -, δ -, and κ -opioid receptor genes constitute a GPCR subfamily, together with a fourth member encoding the orphanin FQ/nociceptin receptor. The last receptor and its endogenous ligand share high structural homology with the opioid receptors and peptides. However, the receptor does not bind opioids and the peptide orphanin FQ/nociceptin shows no affinity toward μ , δ , or κ receptors. All four receptor genes display similar genomic organization, with conserved intron-exon junctions, suggesting a common ancestor gene. These genes have been characterized throughout vertebrates, but cannot be identified earlier in evolution. The four genes are referred as to *Oprm1* (μ), *Oprd1* (δ), *Oprk1* (κ), and *Oprl1* (orphanin FQ/nociceptin) (genomic information is available on the NCBI website). The encoded receptors are classically named MOR, DOR, KOR, and ORL-1, respectively, throughout the literature. A novel nomenclature MOP (μ), DOP (δ), KOP (κ), and NOP (orphanin FQ/nociceptin) has recently been proposed by the International Union of Basic and Clinical Pharmacology (IUPHAR) to unify receptor abbreviations. Many single-nucleotide polymorphisms have been identified in both coding and regulatory sequences of human opioid receptor genes, and large genetic investigations are underway to associate haplotypes with neurological or psychiatric diseases. At present, coding

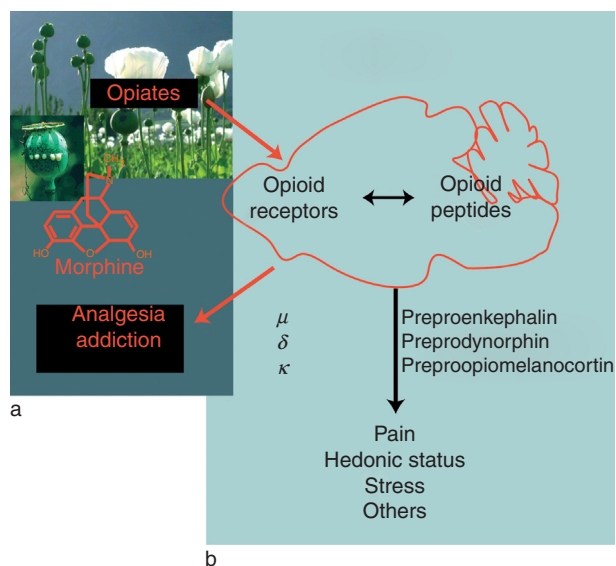


Figure 1 The opioid system: (a) exogenous opiates activate opioid receptors; (b) the endogenous opioid system. In (a), morphine is the main active ingredient of opium and has strong analgesic and addictive properties, which result from a direct interaction of the drug with opioid receptors. In (b), the endogenous opioid system is a neuromodulatory system. The three opioid receptors naturally interact with a family of opioid peptides, produced by proteolytic cleavage of three large precursor proteins. Receptors and peptides are broadly expressed throughout the nervous system and control nociceptive pathways (pain), mood, well-being (hedonic status), and responses to stress. The system also controls respiration, gastrointestinal motility, and endocrine and immune functions.

polymorphisms as well as silent or noncoding polymorphisms that may influence receptor expression levels in the brain are under study.

Physiology of the Opioid System *In Vivo*

Opioid peptides and receptors are strongly expressed in the limbic system, as well as in nociceptive pathways and along the hypothalamic–pituitary–adrenal axis. In these neural circuits, the opioid system regulates addictive and emotional behaviors, and controls responses to pain or stress.

Pharmacology

Three decades of pharmacology have demonstrated an analgesic activity for all three μ , δ , and/or κ agonists. Generally, opioid analgesics used in the clinic are μ agonists. These compounds show strongest efficacy in the treatment of severe acute pain, but their activity remains variable in situations of chronic pain. κ agonists are potential candidates in the treatment of visceral pain. Significantly, studies of μ and κ compounds have highlighted opposing activities of μ and κ agonists on mood. μ agonists show potent

rewarding properties and abuse liability, whereas κ agonists are strongly dysphoric. The latter activity has hindered the development of centrally acting κ agonists for pain control in the clinic. The pharmacology of δ agonists has shown slower progress. The notion that δ agonists may represent useful analgesics with low abuse liability has been proposed for many years and is currently being explored.

Genetics Approach

For a decade, mice lacking μ , δ , or κ receptors, as well as preproenkephalin, preprodynorphin, or β -endorphin, have been created by gene-targeting (knockout mice). Single mutant mice, or even the triple receptor knockout mice, are viable and fertile and show no obvious developmental deficit, indicating that the opioid system is not essential for survival. These mutant mice have been extensively analyzed either for spontaneous behaviors or in response to opioid and nonopioid drugs. Parallel to the pharmacology, this genetic approach has clarified the specific contribution of each molecular component of the opioid system in opioid-controlled physiology and behaviors *in vivo*. In particular, the comparative analysis of μ , δ , and κ knockout mice has clearly shown very distinct activity patterns for each receptor *in vivo* (Figure 2). Similar comparisons are currently underway for peptide knockout mice. Main conclusions from gene knockout mice are the following.

μ receptors represent the primary molecular target for morphine *in vivo* and mediate both beneficial and adverse effects of the most broadly used opiate. μ receptors also mediate the rewarding properties of nonopioid drugs of abuse including cannabinoids, alcohol, and nicotine or even natural reinforcers such as social interactions. μ receptors therefore represent a key molecular trigger for reward, and most likely contribute to the initiation of addictive behaviors. κ receptors, as predicted by the pharmacology, mediate dysphoric activities of both κ opioids and cannabinoids and oppose μ receptors in regulating the hedonic tone. δ receptors are less directly involved in hedonic control. Very distinct from μ and κ receptors, δ receptors regulate emotional responses and show anxiolytic and antidepressant activity. This specific function of δ receptors is now confirmed both by gene knockout and pharmacological studies using SNC80, the only commercially available highly selective δ compound. Further analysis of δ knockout mice and the development of more selective compounds will probably reveal other activities of delta receptors.

μ , δ , and κ receptor-deficient mice all exhibit enhanced pain sensitivity. This indicates that the three receptors, activated by endogenous opioid peptides, tonically inhibit nociceptive responses. Noticeably,

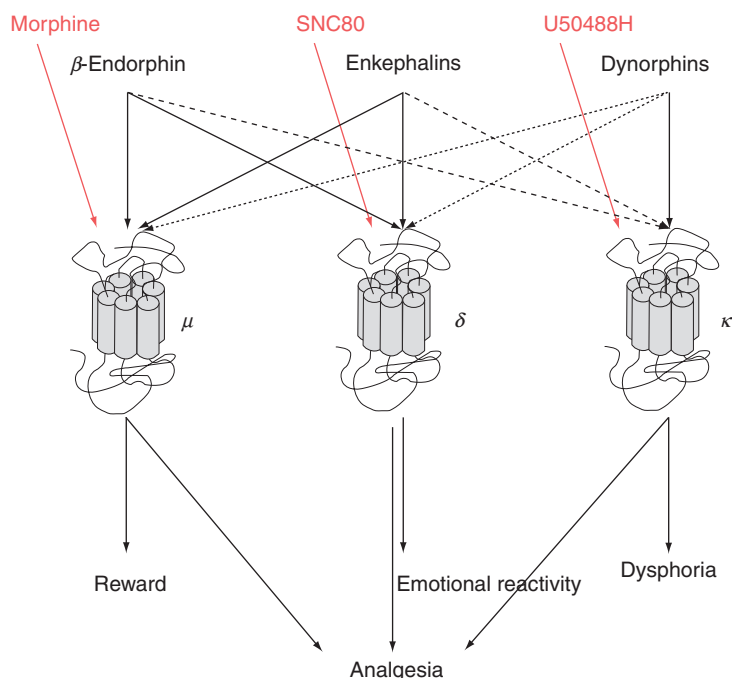


Figure 2 Physiological role of opioid receptors and peptides *in vivo*. Opioid peptides show some, although weak, selectivity toward μ , δ , and κ receptors. A highly selective exogenous (morphine) or synthetic (SNC80 and U50488H) agonist is indicated for each receptor. The combination of pharmacological studies with gene-knockout approaches (targeted gene inactivation in mice) showed that each receptor controls specific neural responses. μ and κ receptors produce euphoria or dysphoria, respectively, whereas δ receptors control levels of anxiety and depressive-like behaviors (emotional reactivity). The activation of all three receptors inhibits pain (analgesia), but each receptor controls a distinct panel of pain modalities.

the phenotypes of mutant mice differ across pain assays. In models of physiological or acute pain, μ receptors modulate mechanical, chemical, and supra-spinally controlled thermal nociception, whereas κ receptors modulate spinally mediated thermal nociception and visceral pain. Again, δ receptors differ from μ and κ receptors in that there is no obvious regulation of acute pain. In contrast, there is evidence for a role of δ receptors in reducing hyperalgesia in situations of inflammatory and neuropathic pain. These data, combined with many pharmacological studies, clearly demonstrate a specific role for each receptor in regulating the broad diversity of pain modalities.

Finally, due to the short half-life and structural similarity of the peptides, identifying a specific role for each endogenous opioid *in vivo* has been difficult. Data from opioid peptide knockout mice do not necessarily follow data from receptor knockouts, consistent with the notion that multiple peptides probably act at each receptor. Although affinity studies and pharmacological approaches suggest a relationship between β -endorphin and μ receptors, enkephalin and δ receptors, and dynorphin and κ receptors (Figure 2), knockout data provide a more complex picture. It is likely that the specific anatomical location of receptors and peptide release largely drive opioidergic synapse functioning.

Receptor Structure

Receptor Binding

Overall μ , δ , and κ receptors show a 60% amino acid sequence identity. Closest homology occurs within the seven-transmembrane helical core, which contains the opioid-binding pocket (Figure 3(b)). Extracellular domains, including three extracellular loops and the N-terminal domain, determine μ , δ , and κ selectivity. These domains differ strongly among receptors and probably act as a gate filtering μ , δ , and κ agonists or antagonists entering the binding pocket. Note that synthetic compounds have been developed with high μ , δ , and κ selectivity, whereas endogenous peptides show limited receptor preference (Figure 2).

Receptor Signaling

As in all GPCRs, opioid receptors convey extracellular signals within the cell by activating heterotrimeric G-proteins, which interact with cytoplasmic domains of the receptor. Agonist binding modifies helical packing of the receptor, and a rearrangement in the positioning of transmembrane domains 3, 6, and 7 has been proposed to drive the transition between inactive and active conformations of the receptor.

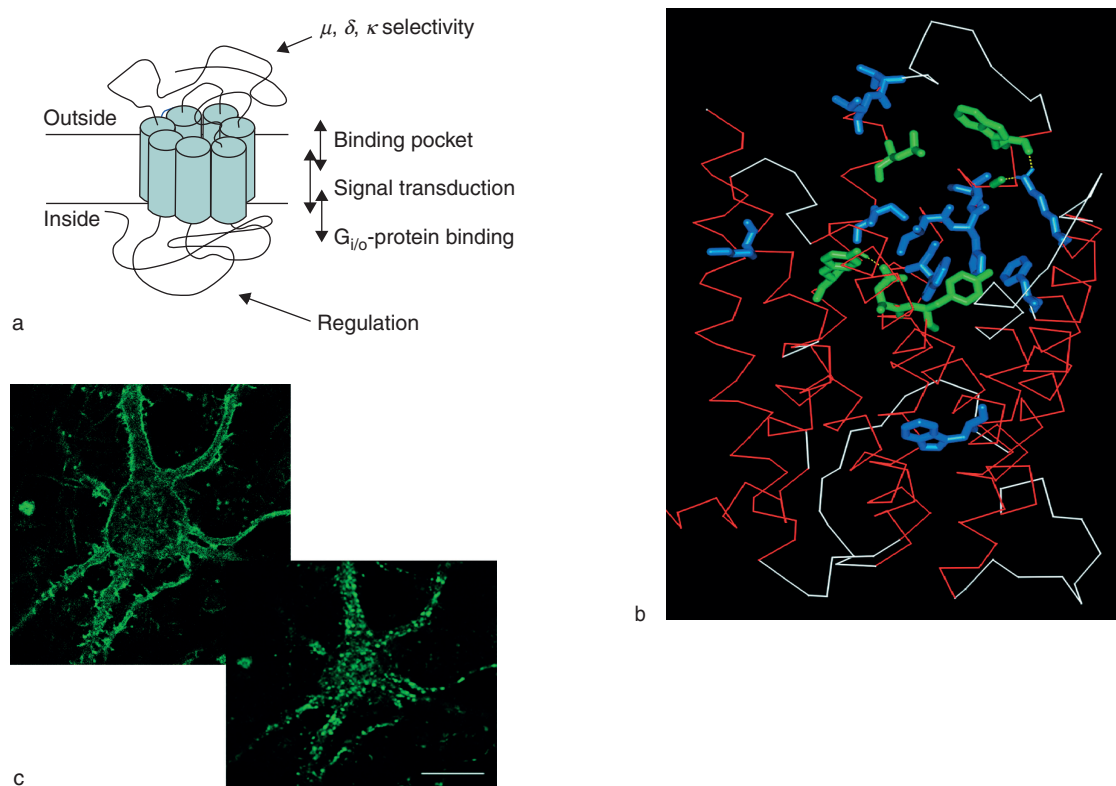


Figure 3 Opioid receptors: (a) receptors belong to the G-protein-coupled receptor superfamily with a seven-transmembrane topology; (b) three-dimensional computer model of the human δ -opioid receptor; (c) direct visualization of δ receptors in native neurons. As shown in (a), μ , δ , and κ receptors are highly homologous. Each receptor domain contributes to receptor function, as indicated. Regulation occurs immediately after the receptor is activated by an opioid agonist and includes receptor phosphorylation, uncoupling from the G-protein, and internalization. In (b), the seven-transmembrane helices are shown in red, and portions of extrahelical loops are in white. The binding pocket penetrates halfway within the helical bundle, and side chains of amino acid residues forming the main binding site are shown in blue and green. As shown in (c), hippocampal neurons extracted from a genetically engineered mouse endogenously produce fluorescent δ -opioid receptors. The receptor is observed at the surface of both cell bodies and processes of the neuron (see continuous green fluorescence on the plasma membrane, top). Exposure to the agonist produces receptor internalization, visible under the form of highly fluorescent dots within the neuron. Note the concomitant fading of surface fluorescence (bottom).

This helical movement modifies the receptor's intracellular structure, hence the receptor–G-protein interaction. Intracellular loops of the receptor form a large part of the receptor–G-protein interface. These intracellular receptor domains are almost identical across μ , δ , and κ receptors, consistent with the fact that all three receptors interact with inhibitory G-proteins of the G_o/G_i type. G-protein subunits dissociate from the activated receptor and, in turn, modulate intracellular effectors and pathways.

Several opioid-evoked signaling events have been identified both in transfected cells and native tissues. Opioids inhibit voltage-dependent Ca^{2+} channels or activate inwardly rectifying potassium channels, thereby diminishing neuronal excitability. Opioids also inhibit the cyclic adenosine monophosphate (cAMP) pathway and activate mitogen-activated protein kinase (MAPK) cascades, both of which affect cytoplasmic events and transcriptional activity of the cell. Overall, opioids inhibit neurons by decreasing

either neuronal firing or neurotransmitter release, depending on the post- or presynaptic localization of the receptors. Finally, opioid receptors are expressed on both excitatory and inhibitory neurons and can therefore exert inhibition or disinhibition within neural circuits.

Regulation of Receptor Signaling

The C-terminal domain differs largely across opioid receptors. Together with intracellular loops, this receptor domain contributes to receptor–G-protein coupling. Significantly, both the C-terminus and intracellular loops also interact with other cellular effectors following receptor activation. Effectors include receptor-specific (G-protein-coupled receptor kinase (GRK)) or-nonspecific (protein kinase A (PKA) and protein kinase C (PKC)) kinases, arrestins that act as adaptors between the receptor and the endocytic machinery, and other recently identified proteins.

These interacting proteins drive the desensitization of receptor signaling, which typically occurs as an adaptive response after receptor stimulation. Desensitization mechanisms include receptor phosphorylation and uncoupling from the G-protein, as well as receptor endocytosis (Figure 3(c)) and redistribution between the cell surface and intracellular compartments. Largely determined by C-terminal epitopes, these dynamic phenomena differ across μ , δ , and κ receptors. Many studies have addressed the mechanisms of opioid receptor trafficking and desensitization in cellular models and, more recently, *in vivo*. At present, studies on native neurons suggest that agonist-stimulated δ receptors are mainly targeted toward degradation pathways after endocytosis, whereas internalized μ receptors are resensitized in early endosomes and rapidly recycle back to the cell surface. Understanding the dynamic aspects of receptor trafficking to and from the cell surface has become a major field in GPCR research.

Chronic Opiates

Regulatory events that occur at the receptor level, as already described, represent adaptive mechanisms that classically contribute to cellular homeostasis under acute agonist stimulation. Chronic exposure to opiates, however, has long-term consequences, which lead to strong and perhaps irreversible alterations of brain functioning at the cellular, synaptic, and network levels. Behaviorally, these adaptations are manifested by tolerance, defined as a reduced sensitivity to the drug effects, and dependence revealed by drug craving and withdrawal symptoms. Many efforts have been made for several decades to understand the molecular basis of these elaborate responses to chronic opiates. Modifications of opioid receptor-associated ion channels and second-messenger pathways have been demonstrated in several brain areas. Also several protein kinases, including PKA, PKC, and calcium/calmodulin-dependent protein kinase (CaMK)II; glutamate receptors; cytoskeleton proteins or neurotrophic factors are involved. Finally opposing neurotransmitter systems – called anti-opioid systems – are recruited. Integrating these observations into a coherent explanation for tolerance and dependence *in vivo* remains a challenge.

Opioid Receptor Heterogeneity

The Existence of Opioid Receptor Pharmacological Subtypes

Opioid receptor pharmacology is complex and the existence of multiple μ , δ , and κ receptor types has

been proposed since the early 1970s. Gene cloning led to the characterization of only three receptor genes, and the molecular basis for pharmacological diversity has long remained a matter of debate. Alternative splicing has been reported, but it has been extremely difficult to establish the biological relevance of these alternative transcripts *in vivo* and to correlate their existence with the multiple opioid receptor subtypes that were described earlier by the pharmacology. Today, it is admitted that the three main Oprm1-, Oprd1-, and Oprk1-encoded receptors are highly dynamic proteins, which may indeed account for the wide diversity of pharmacological subtypes.

Three Receptor Proteins, Many More Cellular Responses

There are several ways to explain pharmacological heterogeneity of opioid receptors. First, increasing evidence supports the notion that μ , δ , and κ receptors may adopt multiple active conformations. Mutagenesis data suggest the existence of multiple binding modes for opioids within the binding pocket. Further, signaling studies in cellular models show that receptor activation and subsequent regulations are strongly drug-dependent. Hence the ligand–receptor complex, rather than the receptor itself, determines the ultimate physiological cellular response. A second source of heterogeneity is the direct cellular environment of the receptor. Heterotrimeric G-proteins differ across cell types, and the number of possible G-protein-associated signaling pathways has expanded dramatically. The variable combinations of G-protein subunits and the nature of associated signaling networks necessarily generate neuron-specific, or even neuron compartment-specific, responses. Also, many regulatory proteins directly interact with the receptor C-terminal tail (as already discussed) and potentially influence opioid-receptor pharmacology, as was demonstrated for several other GPCRs. A third potential receptor modulator is another receptor molecule. The possibility that GPCRs exist as dimeric or oligomeric complexes has gained evidence in the recent years. Co-expression data suggest that the physical association of opioid receptors either as homodimers or heterodimers, or even with other GPCRs, creates novel receptor entities with unique pharmacological properties, which increases opioid receptor heterogeneity. Whether receptor dimerization truly modulates opioid pharmacology *in vivo* remains an important question.

In conclusion, molecular approaches have provided a novel view of opioid receptors. The receptor is now considered a dynamic multicomponent unit rather than a single protein entity. It is likely that the complexity of opioid responses will extend beyond the previously

reported pharmacological subtypes as *in vivo* molecular pharmacology develops. Rational design of opioid compounds that activate a specific subset of μ , δ , or κ receptor-associated signaling pathways is a possible strategy for developing novel drugs of high therapeutic value and low adverse activities, but this still remains a far distant goal.

Conclusion

The last decade in opioid research has led to a better understanding of the genetic basis of opioid-controlled behaviors and physiology. It has also opened avenues toward the development of novel therapeutic opioids in the field of chronic pain, emotional disorders, and addictive diseases. Recombinant technologies have allowed high-throughput screening for novel opiate compounds, the detailed molecular analysis of receptor structure and function, and the identification of associated proteins regulating receptor signaling.

Ongoing studies have returned to analyzing opioid receptor and peptides operating in their physiological environment, using molecular and genetic tools as well as high-resolution imaging techniques. There is a need to identify neural sites where opioid peptide release and subsequent opioid receptor activation controls nociceptive, emotional, and motivational circuits. Also, it is important to characterize signaling pathways that are relevant to specific behavioral or physiological responses to opioids *in vivo*. In addition, novel insights into brain function will arise from understanding the interactions of the opioid system with other neurotransmitter systems. For example, whether the opioid system interacts with the cannabinoid system or anti-opioid systems at the molecular, cellular, or circuit level remains an open question. Last, the elucidation of molecular and network adaptations to chronic opiates will undoubtedly shed light on the general mechanisms of brain plasticity.

See also: Addiction: Neurobiological Mechanism; Drug Addiction: Behavioral Neurophysiology; Invertebrate Neurohormone GPCRs; Neuropeptides: Pain; Single Photon Emission Computed Tomography (SPECT): Technique.

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