

Chapter 1

Opioid receptors and opioid pharmacodynamics

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Introduction

Opioids have a long and rich pharmacology. They are widely used throughout medicine and have been invaluable. However, they come with problems, including side effects such as constipation, respiratory depression, and sedation, as well as the potential of abuse. Investigators have long believed that more selective drugs lacking these drawbacks could be developed, leading to the synthesis of novel analgesics that have proved to be extraordinary tools for the early pharmacologists in the field (Fig. 1.1). The structure–activity relationships for the vast number of agents led to the conclusion that there must be specific recognition sites or receptors for these drugs,^{1–3} going so far as to suggest specific molecular interactions between the ligand and its binding pocket. However, the biochemical demonstration of these receptors had to wait until 1973.^{4–7} These binding sites were highly selective for opioid analgesics and their antagonists, and demonstrated the same structure–activity relationships seen pharmacologically, including stereospecificity. Much of the work in the past 30 years has focused on identifying, characterizing, and, most recently, cloning these receptors, and correlating them with opioid action.

Opiates and opioid peptides

The original opiates, morphine and codeine, are derived from opium. Over the years, thousands of derivatives were synthesized in an effort to dissociate analgesia from problematic side effects, particularly respiratory depression, constipation, and dependence liability. Morphine has a rigid structure (Fig. 1.1). Systematic studies showed that a number of modifications of the morphine structure could be tolerated without losing analgesic activity, including eliminating significant portions of the molecule. For example, elimination of the C ring provided the framework for the benzomorphans, such as ketocyclazocine, ethylketocyclazocine, and pentazocine. Although these ligands retained analgesic activity, their pharmacology was quite distinct from morphine and led to the identification of kappa receptors⁸ long before the discovery of their endogenous ligand, dynorphin A.^{9,10} However, further simplification of the structure led to other opioids with actions more similar to those of morphine, including methadone, pethidine (meperidine) and the fentanyl series of mu opioids¹¹ (Fig. 1.1).

The strict structure–activity relationships of the opioids followed by the demonstration of their receptors clearly indicated that there must be an endogenous ligand for these sites. The first physiological evidence for the presence of endogenous opioids came from the studies of Liebeskind and coworkers, who demonstrated analgesia following stimulation of the peri-aqueductal grey, an action that was reversed by the opioid receptor antagonist naloxone.^{7,12,13} The isolation of opioid-like materials from the brain^{14–17} led to the determination of the structure of the

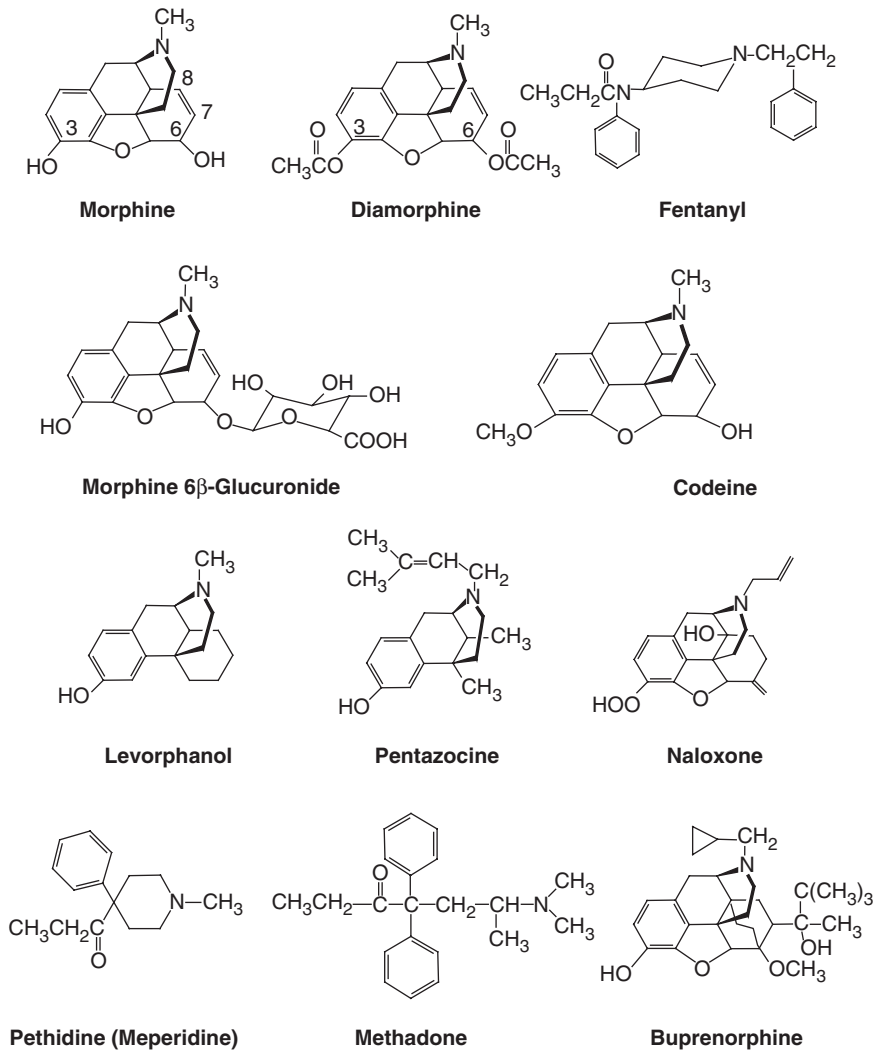


Fig. 1.1 Structure of common opioids.

enkephalins, β -endorphin, and the dynorphins (Table 1.1).^{18,19} The term endorphins was proposed to encompass all the endogenous opioids, with the enkephalins referring to the two pentapeptides first identified by Kosterlitz⁷ (Table 1.1).

Goldstein then described the dynorphins, a series of peptides that shared the same first five amino acids as the enkephalins and had high affinity for kappa receptors.^{7,9,10} Although all these endogenous peptides display affinity for the three main opioid receptors, dynorphin A binds preferentially to kappa opioid receptors and the enkephalins to delta opioid receptors. The endogenous ligand for the mu receptors is still not entirely clear, although the endomorphins label this site with high affinity and specificity.²⁰

The endogenous opiates are derived from a family of three precursor proteins that are processed to generate the various peptides.²¹ The discovery of these independent precursors firmly established the independence of these opioid peptides from each other. Thus, the

Table 1.1 Structure of some common opioid peptides

<i>Natural opioid peptides</i>	
[Leu ⁵]enkephalin	Tyr-Gly-Gly-Phe-Leu
[Met ⁵]enkephalin	Tyr-Gly-Gly-Phe-Met
Dynorphin A	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln
Dynorphin B	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr
α -Neendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys
β -Neendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro
β -Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu
Endomorphin-1	Tyr-Pro-Trp-Phe-NH ₂
Endomorphin-2	Tyr-Pro-Phe-Phe-NH ₂
Orphanin FQ/Nociceptin	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asp-Glu
<i>Synthetic opioid peptides</i>	
DPDPE	[D-Pen ² ,D-Pen ⁵]enkephalin
DADLE	[D-Ala ² ,D-Leu ⁵]enkephalin
DALDA	Tyr-D-Arg-Phe-LysNH ₂
DAMGO	[D-Ala ² ,MePhe ⁴ ,Gly(ol) ⁵]enkephalin
DSLET	[D-Ser ² ,Leu ⁵]enkephalin-Thr ⁶
Deltorphin I	Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH ₂
CTOP	D-Phe-c[Cys-Tyr-D-Trp-Orn-Thr-Pen]-Thr-NH ₂

enkephalins were not simply breakdown products of dynorphin and β -endorphin. The dynorphins are produced from preprodynorphin, while the enkephalins are generated from a distinct precursor protein, preproencephalin. β -Endorphin is a 31-amino-acid peptide that also possesses potent opioid activity,²² but what makes it unique is its localization to the pituitary gland and the fact that it is produced from the same precursor protein (β -lipotropin) that generates adrenocorticotrophic hormone (ACTH) and other hormones. Furthermore, it is co-released into the blood with ACTH as part of the stress response, perhaps contributing to the role of stress to modulate pain perception.²³ Thus, the opioid peptides represent a family of highly related neurotransmitters.

Antagonists

Antagonists have been extremely valuable, both clinically and in studies of opioid mechanisms. Antagonists such as naloxone effectively reverse the actions of traditional opioids. However, their clinical use is complicated by their ability to precipitate withdrawal in dependent subjects. Thus, care must be taken when administering an antagonist to a subject who has been on opioids chronically, particularly since sensitivity towards an antagonist progressively increases as the subject becomes more physically dependent. Although naloxone is used clinically to reverse opioid actions, its duration of action is relatively brief, with the actions of many agonists lasting longer. Thus, care must be taken to re-administer it when counteracting longer-acting agonists.

Although agonists and antagonists bind to the same receptor, they interact with different conformations of the protein which can be influenced biochemically in a variety of ways, including monovalent and divalent cations,^{24–26} enzymes and protein-modifying treatments,^{27–29} and guanine nucleotides.³⁰ Our understanding of how these different conformations actually bind their ligands is now being explored at the molecular level *in silico* using detailed computer modelling approaches. However, it will require biochemical approaches to define these issues experimentally.

Antagonists have proved valuable in studies of pharmacological mechanisms. They can verify the opioid nature of a response, and the availability of highly selective antagonists has greatly facilitated our understanding of opioid receptor multiplicity. Selective mu antagonists, such as β -funaltrexamine,^{31,32} have proved valuable in defining mu actions both *in vivo* and *in vitro*, while other agents, such as naloxonazine, have demonstrated subpopulations of mu receptors.^{33–36} Naltrindole is an excellent delta receptor antagonist, whilst nor-binaltorphimine selectively blocks kappa₁ receptors.^{37,38}

Partial agonists

Unlike full agonists, partial agonists have limited intrinsic activity at the receptor, a measure of the activation of the receptor by the drug. Depending upon the situation, drugs with limited intrinsic efficacy may not achieve a complete response at full receptor occupancy. Clinically, this may lead to a ceiling effect, in which further increases in drug dosing will not further increase the analgesic response. The ability to produce a full response, termed efficacy, depends on the situation in which it is measured. For example, partial agonist opioids that are efficacious in low intensity pain models may be unable to provide a complete response with more intense pain intensities, as shown by a plateau in the response that cannot be overcome with further increases in dose. There may also be a shift of the dose–response curve to the right, so that the higher drug doses needed to relieve the pain also increase dose-limiting side effects.

It is possible to assess the intrinsic activity and efficacy of drugs at the molecular level. Earlier studies utilized receptor-binding approaches to assess the agonist/antagonist character of the drugs.^{24,25} However, this approach has a number of limitations. Functional approaches are more direct. The most straightforward involves looking at the activation of G-proteins. Opioid receptors are coupled to G-proteins that are responsible for transducing the response following receptor activation. When agonists bind to the receptor, they initiate the dissociation of guanosine 5-diphosphate (GDP) from the G subunit of the α -subunit of the heterotrimeric G-protein complex, which is then replaced by guanosine 5'-triphosphate (GTP). The G subunit then dissociates, liberating the G $_{\alpha}$ and the G $_{\beta\gamma}$ subunits which can then interact with downstream transduction systems. The ability of a drug to activate the G-protein depends on its intrinsic activity. Partial agonists, which have low intrinsic activities, activate G-proteins less efficiently, resulting in a lower overall activation at full receptor occupancy. Pure antagonists do not activate the G-protein and have no effect. The ability of drugs to activate the G-proteins can be assessed experimentally by measuring the dissociation of GDP and its replacement by a non-hydrolysable radiolabelled GTP analogue [³⁵S]GTP γ S. Partial agonists are unable to activate as many G subunits as full agonists and thus have lower levels of [³⁵S]GTP γ S binding than full agonists. Comparing the potency (EC₅₀) of an opioid to induce [³⁵S]GTP γ S binding with the binding affinity of the ligand determined through receptor binding assays (K_i) can provide an indication of intrinsic activity. Antagonists do not induce binding of the GTP analogue.

Inverse agonists are unusual and differ from both agonists and antagonists. Most receptors typically have low levels of constitutive activity, leading to a low level of G-protein activation in the absence of agonist. Antagonists are neutral, neither inducing G-protein activation nor reversing

the constitutive receptor activity. In contrast, inverse agonists block the constitutive receptor activity.

Most of the opioids used clinically are full agonists, but there are a number that are partial agonists at mu receptors. However, most of these drugs also interact with kappa receptors, leading to their classification as mixed agonist-antagonists and making their pharmacology quite complex. Much of their analgesic activity is thought to involve kappa systems, but their partial agonism at mu receptors remains quite important. It is thought by some that this makes them less likely to be abused. However, if they are administered to a patient who is dependent upon a pure mu agonist, these partial agonists can precipitate a full withdrawal syndrome.

Classification of opioid receptors

Opiates and the opioid peptides act through a family of receptors. Classical pharmacological studies indicated the presence of multiple classes of opioid receptors long before their identification biochemically. Based upon the interactions of nalorphine and morphine in clinical studies, Martin proposed distinct receptors for the two drugs over 35 years ago,³⁹ which subsequently led to the current classification of mu and kappa receptors.⁸ Delta receptors, selective for the enkephalins, were identified in bioassays and then biochemically.⁴⁰ Initially, opioids were classified by their actions in bioassays. The guinea pig ileum bioassay is relatively selective for mu opioids, while delta drugs were defined by their actions in the mouse vas deferens bioassays. However, many of these assay systems actually contain more than one class of receptor, and drugs are currently classified by their affinities against mu, delta, and kappa receptors in traditional receptor binding assays.

Mu receptors display high selectivity for morphine and related synthetic compounds. Identification of the endogenous ligand for the mu receptor has proved difficult. Many of the endogenous opioids, particularly β -endorphin, have reasonably high affinity for mu sites, but the most selective of the endogenous ligands are the endomorphins. The structural requirements of the synthetic compounds for affinity for mu receptors are met by a broad range of opiates, ranging from the rigid structure of morphine to flexible structures like methadone and fentanyl, and even synthetic enkephalin derivatives.¹¹ Kappa receptors were initially proposed, based upon the actions of ketocyclazocine⁸ before the discovery of their endogenous ligand, dynorphin A.^{9,10} Delta receptors were then proposed, based upon their selectivity for the enkephalins.^{41,42} The pharmacology of these receptor families has been facilitated by the synthesis of highly selective agonists for all of them.

Pharmacological evidence from a number of laboratories has suggested subtypes of these receptors. Within the mu opioid receptor family, the ability of the highly selective mu antagonist naloxonazine to dissociate supraspinal morphine analgesia from both respiratory depression and the inhibition of gastrointestinal transit, coupled with binding studies, led to the suggestion of mu₁ and mu₂ receptor subtypes.^{33,34,43-49} The possibility of distinct mu receptors for morphine-6-glucuronide then arose.^{50,51} Mu receptor multiplicity has now been confirmed at the molecular level through the cloning of a number of mu opioid receptor splice variants.⁵²⁻⁵⁷

Multiple kappa receptors also have been proposed, starting with the initial suggestion of kappa₂ by Zukin and colleagues.⁵⁸ This was then extended to kappa₃ receptors, based upon the pharmacology of a novel opioid naloxone benzoylhydrazone.⁵⁹⁻⁶⁴ Finally, computer modelling of binding data suggested the presence of subtypes, even with the kappa₁ receptor classification,^{64,65} with one being highly sensitive to the endogenous opioids dynorphin B and α -neoendorphin.⁶⁴ The two kappa₁ receptor subtypes are both sensitive to the kappa₁-selective drug U50,488H, whereas the kappa₂ and kappa₃ receptors are not. Finally, pharmacological studies have also led to the suggestion of delta receptor heterogeneity,⁶⁶⁻⁶⁹ although these have not yet been identified at the molecular level.

Anatomical localization of opioid receptors

Opioid receptors have been demonstrated throughout the nervous system, from peripheral nerves to the spinal cord to the brain. Peripheral opioid receptors are synthesized in the dorsal root ganglion, and transported to nerve endings and centrally to the dorsal horn of the spinal cord. Axonal opioid transport is enhanced by inflammation.^{70,71} Although the opioid receptors have been localized to regions sensitive to opioid action, they are also present in many sites unrelated to pain modulation. Furthermore, many of the immune cells associated with an inflammatory response synthesize endogenous opioids, including enkephalins,⁷⁰ and contain opioid receptors, which may help explain the utility of peripheral opioids for inflammatory conditions and the clinical use of topical opioids in open wounds and within joints.^{72–74}

All three classes of opioid receptors are localized with high density within the superficial layers (lamina I and II) of the spinal cord, with lower levels in the deeper lamina. Within the dorsal horn, mu receptors are the most dense, accounting for 70% of the receptors, followed by delta receptors (24%) and kappa₁ (6%).^{75–78} These receptors are localized both pre- and post-synaptically.⁷⁷

Supraspinally, mu opioid receptors are found within the amygdala and nucleus accumbens, regions associated with the reinforcing behaviour of opioids, as well as the striatum, which is important in motor control. Their functional significance within the motor systems is not clear, but they are important mediators of the analgesic responses within the limbic system, which is important in the emotional components of pain. Within the thalamus, mu opioid receptors are more prominent within the medial structures than within the lateral ones. The medial thalamic nuclei relay spinothalamic input from the spinal cord to the cingulate gyrus and limbic structures.⁷⁵ Mu receptors also have a well-established distribution within the brainstem, with high densities in a number of structures associated with analgesia, including the peri-aqueductal grey, the reticular formation, the locus coeruleus, and the rostral ventromedial medulla.^{70,79,80} The peri-aqueductal grey, the locus coeruleus, and the rostral ventromedial medulla are responsible for a descending modulatory system, which dampens or facilitates dorsal horn pain processing. Opioid receptors are also found within the hypothalamus, where they are presumed to be involved with hormonal regulation, as well as the medullary vagal complex, the nucleus tractus solitarius, and the area postrema. These locations mediate the endocrine and autonomic actions of opioid, as well as nausea.^{79–82}

The distributions of the delta and kappa receptors also have been described. Although they demonstrate similar distributions within the spinal cord, their supraspinal distributions differ.^{79,83,84}

Behavioural opioid actions

Analgesia

The utility of morphine and related drugs rests with their ability to modulate pain. They differ in many respects from other pain drugs. First, pure opioid agonists do not display a ceiling effect. Thus, increasing the dosage will continue to increase pain relief, although side effects commonly interfere with dose escalation. Partial agonists may show ceiling effects in selected circumstances, depending upon the nature and intensity of the pain, and mixed agonist–antagonist drugs must be used cautiously in patients previously treated with opioids. In contrast, other analgesics, such as the non-steroidal antiinflammatory drugs (NSAIDs), all display ceiling effects. The opioids act upon the subjective ‘hurt’ associated with pain without affecting primary sensory modalities. This distinguishes them from local anesthetics, which interfere with all sensory input and, at sufficiently high concentrations, motor function as well.

Table 1.2 Systemic /spinal opioid synergy

Morphine route	Morphine ED ₅₀	Morphine ED ₅₀ (95% confidence limits)	Systemic shift
Systemic alone	3.1 mg /kg	(1.6, 4.4)	
Intrathecal alone	305 ng	(153, 501)	
Systemic +25 ng, i.t.	0.5 mg /kg	(0.4, 0.8)	6.2
+50 ng, i.t.	0.3 mg /kg	(0.2, 0.5)	10.3
+100 ng, i.t.	0.2 mg /kg	(0.1, 0.3)	15.5
+200 ng, i.t.	0.037 mg /kg	(0.01, 0.10)	83.8

Morphine analgesia was assessed in groups of mice and the ED₅₀ determined following systemic administration alone, intrathecal administration alone and for systemic administration with the indicated fixed dose of intrathecal morphine. Administration of low doses of morphine intrathecally that are insufficient to produce an analgesic action alone are still capable of potentiating the activity of systemic morphine. Results are from the literature.⁸⁷

Opioid analgesia is mediated within both the central and peripheral nervous systems. Numerous sites of action have been mapped in the brainstem, including the periaqueductal grey, the nucleus raphe magnus, and the locus coeruleus, as well as in the dorsal horn of the spinal cord,⁸⁵ regions known to have high levels of opioid receptors. In the periphery, opioid receptors have been demonstrated on peripheral nerves, and peripheral opioids have clear analgesic actions. Although each site is important and can elicit an analgesic response independent of the others, simultaneous activation of more than one site results in synergy. First demonstrated with spinal and supraspinal morphine,⁸⁶ synergistic interactions have also been documented between the periphery and the central sites^{87,88} and even among brainstem nuclei.⁸⁹ These regional interactions are important because systemic drugs simultaneously activate all sites.

Epidural opioids present a unique clinical situation. The instillation of opioids epidurally leads to high levels at the spinal level because of diffusion into the subarachnoid space. However, epidural drugs also have an appreciable systemic absorption. Animal models demonstrate that even low doses of intrathecal morphine will dramatically potentiate the analgesic response of systemic morphine, shifting the systemic dose–response curve 10-fold or more to the left (Table 1.2). Thus, the synergy due to the combination of elevated opioid levels spinally with the systemically absorbed drug may help to explain the utility of this approach. Since intrathecal opioids have reduced systemic absorption, their actions may be more localized spinally.

Kappa and delta drugs also display analgesia peripherally, spinally, and supraspinally. However, their overall clinical utility is far less than mu systems because of the limited availability of selective opioids. Indeed, the kappa drugs currently available, particularly pentazocine and nalbuphine, are mixed agonist–antagonists with strong mu antagonist actions. There are not yet any clinically useful delta opioids.

Pharmacogenomics and opioid analgesia

Although virtually all strains of mice respond to opioids, there are intriguing genetic differences in sensitivity. The genetics of opioid sensitivity has been extremely well studied in a number of laboratories.^{90–92} However, a simple study examining the responses of different strains of mice to a fixed dose of morphine mimics the clinical situation (Table 1.3). Following a fixed morphine dose, the responses of various strains of mice ranged from 80% to 0%.⁹³ The genetic variability among

Table 1.3 Sensitivity of mouse strains to morphine

Strain	Morphine analgesia
BALB/c	90%
CD-1	76%
C57/bg ^J	62%
HS	62%
Swiss Webster	40%
C57/+	40%
CXBK	0%

Groups of mice ($n \geq 10$) from the indicated strain received a single dose of morphine (5 mg/kg, s.c.) and analgesia tested 30 min later using the radiant heat tailflick assay. Analgesia was defined as a doubling or greater of the baseline tailflick latency. Adapted from the literature.⁹³

these strains is arguably no different than what we encounter with patients. Thus, it is not surprising that the responses of patients to a drug can differ.

However, these genetic differences are even more complex, as illustrated by comparisons of the CD-1 and CXBK strains (Fig. 1.2). Doses of a series of mu opioids were chosen that elicited similar analgesic actions in CD-1 mice and were then given to the CXBK mice. Although the CXBK mice were not very sensitive to morphine, they responded normally to several other mu opioids, including methadone and heroin.^{94,95} Clearly, there are differences among the mu opioids in this animal model, much as we see in the clinical situation where the responses of individual patients to different mu opioids can vary.

Analgesic tolerance

Chronic opioid use leads to a progressive decline in potency, a phenomenon termed ‘tolerance’. Put another way, with continued usage, the dose of opioids must be increased to maintain a fixed

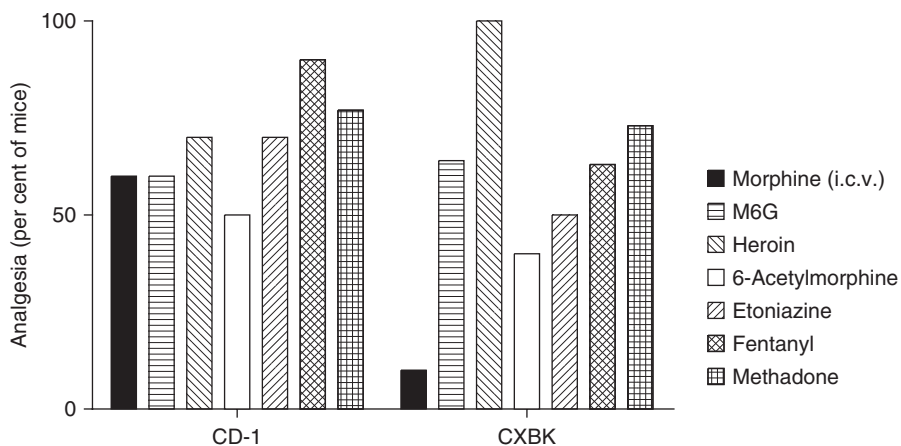


Fig. 1.2 Analgesic activity of opioids in CD-1 and CXBK mice. Doses of the indicated drugs were chosen to give similar analgesic actions in CD-1 mice and then were administered to the CD-1 mice. From the literature.^{94,95}

response. Tolerance is due to a wide variety of responses, ranging from biochemical changes at the receptor to more generalized changes within *N*-methyl-D-aspartate (NMDA) neuronal circuits. The roles of other neurotransmitter systems, including NMDA receptors and nitric oxide, are particularly interesting, since inhibitors of nitric oxide synthase and NMDA receptor antagonists can diminish or reverse tolerance in animal models.^{96–101}

Cross-tolerance implies that subjects tolerant to one opioid will be tolerant to another and is limited to drugs acting the same receptors. Thus, animals tolerant to mu opioids do not show cross-tolerance to kappa or delta drugs. Complete cross-tolerance implies identical receptor mechanisms of action, while incomplete cross-tolerance suggests some differences. Within the mu opioid family, preclinical studies show both complete and incomplete cross-tolerance (Fig. 1.3). In mice made tolerant to morphine, codeine shows complete cross-tolerance but a number of other mu opioids, including methadone and heroin, do not.¹⁰² These preclinical studies are similar to observations made with patients who often show incomplete cross-tolerance, helping to explain the utility of opioid rotation.¹⁰³

Opioid dependence

Dependence is a physiological response to chronic administration of opioids. It has been most closely studied with mu opioids. Withdrawal refers to the signs and symptoms seen with the abrupt discontinuation of the opioid or the administration of an antagonist. On the street, it is termed going ‘cold turkey’, in part due to the piloerection seen in many patients. It is important to distinguish dependence from addiction. Dependence is a physiological response seen in all subjects maintained on opioids, whereas addiction implies a psychological dependence and is uncommonly seen in patients with no prior history of drug abuse.

Clinically, dependence is not a concern as long as patients continue to take their opioid. However, if their analgesic is withheld, they will undergo withdrawal. Care must be taken with dependent patients, since switching them to a partial agonist or mixed agonist–antagonist, such as pentazocine, will precipitate withdrawal in some situations. Antagonists can precipitate

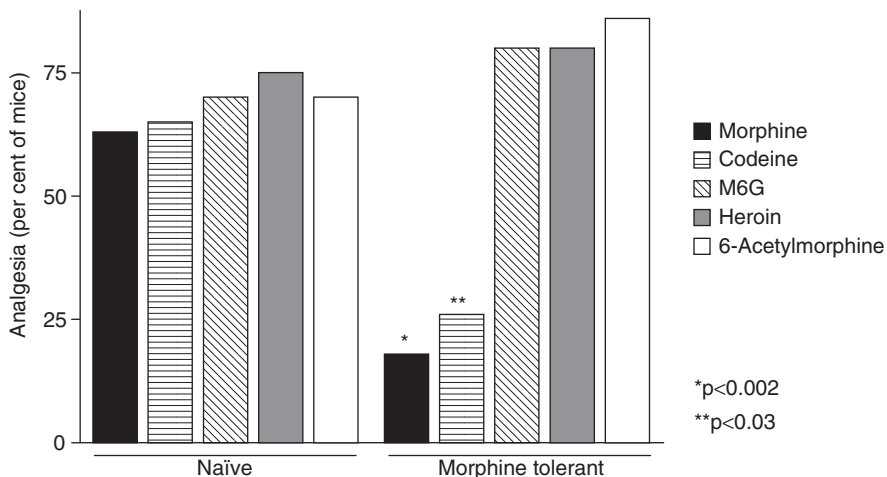


Fig. 1.3 Incomplete cross-tolerance among mu opioids. Doses of the indicated drugs were chosen to give similar analgesic actions in naïve animals. Groups of mice were given morphine daily for 4 days and then tested with the same doses of the indicated drugs on the fifth day. From the literature.⁹⁵

withdrawal within seconds. However, with care it is possible to reverse opioid actions without precipitating withdrawal by diluting the antagonist and slowly titrating it against the patient's signs and symptoms.

Other actions

Morphine and related drugs have a variety of other actions in addition to analgesia, including respiratory depression and the inhibition of gastrointestinal transit, which plays a major role in the constipation seen in most patients.^{102,104,105} Opioids also have a number of neuroendocrine actions, influencing the secretion of prolactin, growth hormone, testosterone, and a variety of other hormones. These actions, like analgesia, are reversed by opioid antagonists, such as naloxone, which confirms that they are opioid receptor mediated. Respiratory depression and the inhibition of gastrointestinal transit are of greatest concern clinically. Although both actions are mediated through mu receptors, there is evidence that these actions can be dissociated from analgesia by several novel mu antagonists, leading to the concept of multiple mu opioid receptor subtypes.^{102,104,105} Thus, it may be possible to develop opioids lacking these undesirable actions. Alternatively, selective delta and kappa drugs might avoid some of these problems. Some highly selective kappa drugs have been examined in humans. Although they displayed analgesia, their clinical utility was impaired by the high incidence of psychotomimetic effects and a profound diuresis. Delta drugs have not been studied in detail clinically.

Molecular opioid actions

Opioid actions can be defined at the level of the receptor, the cell, and in the modulation of circuitry within the nervous system. Much progress has been made in these areas over the past few decades, although it is still difficult to integrate all these various foci of investigation. At the cellular level, the three opioid receptors are inhibitory and prevent the presynaptic release of a number of neurotransmitters. The ability to inhibit the release of acetylcholine was first demonstrated in the guinea pig ileum.^{106,107} Of particular interest were the observations that opioids inhibited the release of glutamate, calcitonin gene related protein (CGRP), and substance P in view of their established roles in pain circuitry and nociceptive transmission⁷⁵ (Fig. 1.4). The substance P within the dorsal horn of the spinal cord originates from dorsal root ganglia neurons as well as from neurons intrinsic to the cord. The receptor for substance P, termed the neurokinin I (NK1) receptor, is present on postsynaptic afferent terminals within laminae I, II, and IX.¹⁰⁸ CGRP, another peptide associated with pain modulation, is released from primary afferents and facilitates the activity of substance P within the dorsal horn.⁷⁶ Glutamate is one of the most important transmitters, with actions throughout the nervous system. There are a number of glutamate receptors, including the ionotropic NMDA and alpha-amino-3-hydroxy-5-methyl-4isoxazolepropionate (AMPA) receptors, as well as the metabotropic glutamate receptors which belong to the G-protein coupled receptor family. Nociceptive transmission is thought to involve all three transmitter systems. Glutamate has a unique place in nociception since activation of NMDA receptors has been associated with centrally mediated chronic neuropathic pain and hyperalgesia and 'wind up', which is induced by sustained depolarization of wide dynamic range (WDR) neurons found in deeper layers of the dorsal horn.^{76,109-112} Opioids modulate both ion channels and transduction systems involved with G-protein coupled receptors.

Electrophysiology

Opioids have a variety of effects on channels and thus on the ionic conductances in the cell. Cyclic adenosine 5-phosphate (cAMP) modulates the membrane 'pacemaker' current that influences

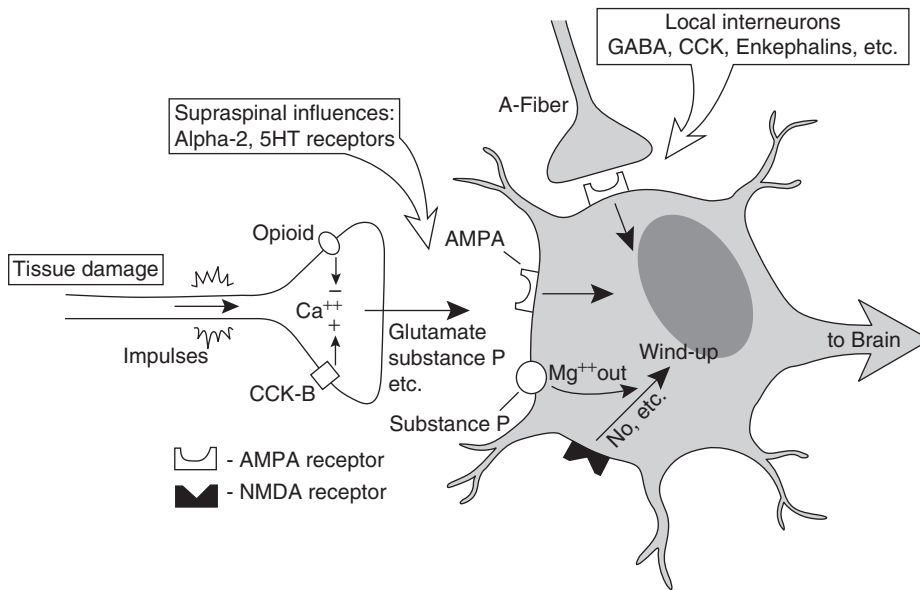


Fig. 1.4 Schematic of neurotransmission between primary afferents and wide dynamic range neurons (WDR).

the excitability of the cell. Opioids inhibit cAMP formation and thereby reduce spontaneous neuronal depolarization by maintaining the membrane in a hyperpolarized state.^{113,114} This, in turn, reduces neurotransmitter release, consistent with the results described above. Opioids also induce membrane hyperpolarization through activation of G-protein activating inwardly rectifying potassium (GIRK) channels.^{75,115} Four GIRK channels have been identified (GIRK I, II, III, and V), of which GIRK-II is particularly important in opioid analgesia.¹¹⁶ High-efficacy opioids maintain GIRK in an active state and produce a longer duration of potassium influx. Overall, the coupling of opioids with GIRK appears to be less efficient than opioid-calcium channel interactions or cyclic AMP inhibition.^{113,114}

Opioids also block voltage-gated calcium channels,^{75,117} presumably through their activation of pertussis-toxin-sensitive G_i and/or G_o proteins. The G subunits of the heterotrimeric G protein are thought to be primarily responsible for voltage-gated calcium-channel blockade.¹¹³ Although calcium channels are blocked by opioids through activation of G_o and by G_i subunits, intracellular calcium can be mobilized through opioid activation of phospholipase C, which is initiated by certain G-proteins such as G_{11} , G_{14} , and G_{16} . Intracellular calcium activates several kinases, which blunt opioid responsiveness through opioid receptor phosphorylation and receptor desensitization.¹¹⁸

Opioid receptors and G-proteins

Opioid receptors are coupled to pertussis-toxin-sensitive inhibitory G-proteins, principally $G_{i/o}$, although there is some evidence for excitatory activity in selected situations^{75,119–121} (Fig. 1.5). Opioid receptors reduce cyclic AMP by blocking adenylyl cyclase (AC) activity, which in turn is responsible for a multitude of actions, including modulation of sodium channel activity.¹¹⁸ G-protein-mediated actions include blocking calcium and GIRK channels, as well as increasing intracellular calcium through membrane phospholipase C and the activation of a number of

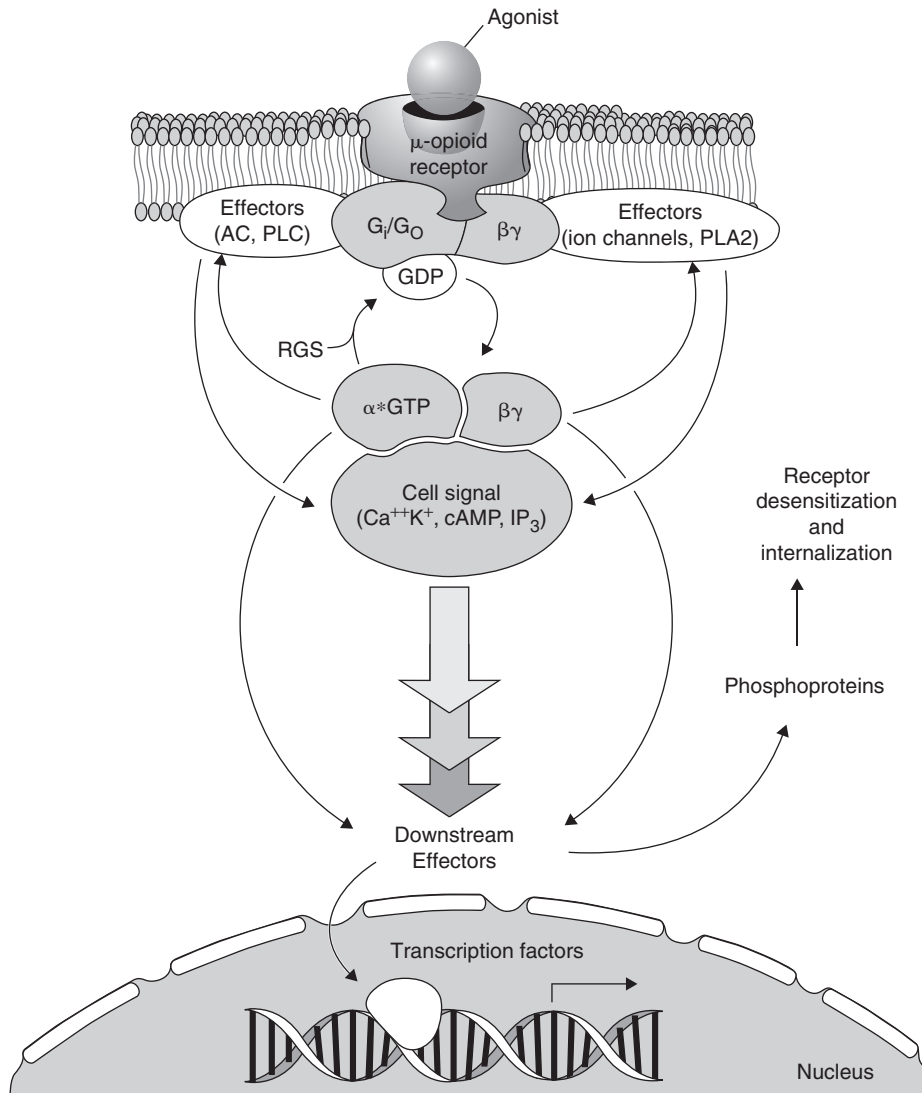


Fig. 1.5 Schematic of interactions between opioids receptors and G proteins.

kinases (mitogen-activated protein kinase or MAPK), which act to balance opioid inhibitory responses.^{118,121}

Opioid receptors are 'promiscuous' in that they can interact with several different G-protein complexes¹¹⁸. The G-protein coupled to the receptor appears to be dependent on the type of opioid receptors, opioid affinity to the receptor, receptor density and availability of G-protein.^{118,121} All three classes of opioid receptors can activate G_{i/o} proteins with equal efficacy, although there are specific preferences for certain G-protein subtypes.^{118,122,123} Opioid receptor G-protein interactions are also tissue and cell specific.¹²⁴ Subtle differences in receptor structural conformation among the three major opioid receptors can influence the type of G-protein coupling specificity.¹²⁴ Opioid receptors will also interact with certain pertussis-toxin-insensitive G-proteins, such as G_Z, G₁₄, and G₁₆, which also stimulate GIRK and inhibit AC.¹¹⁸

One of the paradoxes of opioid pharmacology is the excitatory action associated with the stimulation of adenylyl cyclase activity seen at very low opioid agonist concentrations.^{119,120} At this point, it is not clear whether this effect involves unique receptor subtypes, unusual complements of G-proteins, or adenylyl cyclase isoforms that are stimulated by G subunits G_i and/or G_o.¹¹⁸ The 'superactivation' of adenylyl cyclase produced by the opioids can mimic many of the observations seen with opioid withdrawal.

Regulators of G-protein signalling (RGS) are important modulators of G-protein responses, influencing the duration of G-protein signalling.¹²⁴ RGS proteins serve as GTPase activating proteins, which curtail receptor signalling by facilitating the hydrolysis of GTP to GDP, which in turn leads to the re-association of the G-protein subunits and the termination of their activity. Multiple RGS isotypes which interact selectively with different G-proteins have been described.^{124–126} RGS proteins have also been associated behaviourally with opioid tolerance.¹²⁷

Tolerance

Tolerance is a standard response to continued exposure to opioids, as discussed above. The mechanisms involved with tolerance are extensive, ranging from modifications of the receptor and/or its trafficking, to transduction systems, to general cellular effects and synaptic plasticity, to diffuse changes in circuitry and interactions with other transmitters. Tolerance can be either homologous, in which the effects are limited only to opioid systems, or heterologous, in which similar actions are seen with other transmitters sharing the same effector systems. Desensitization of opioid receptor activity, which some consider a form of tolerance, can occur rapidly following opioid receptor occupation.¹¹⁸ Slower processes, many of which require protein synthesis, include the upregulation of adenylyl cyclase in response to chronic opioids.^{128–130}

It has been suggested that prolonged opioid receptor interactions reduce G-protein coupling.¹³¹ Owing to a combination of desensitization and changes in receptor levels resulting from endocytosis and trafficking, receptor desensitization occurs more rapidly than downregulation, although both are thought to require receptor phosphorylation.¹³² Various kinases, such as G-protein receptor kinases, protein kinase-A (PKA), protein kinase-C (PKC), calcium-calmodulin-dependent protein kinase-II, and tyrosine kinase, can phosphorylate opioid receptors at the third intracellular loop and the C-terminal tail, which is thought to modulate the ability of the receptor to activate the G-proteins.¹³³

Modulation of receptor actions

Opioid function at the cellular level can be influenced by many factors. While all these actions have been clearly demonstrated experimentally, understanding and integrating them into a comprehensive understanding of behaviour has not been easy. The major problem is one of relative importance and causality. These are the major questions to be addressed.

Opioid receptors are internalized after activation by an agonist. Removing the receptor from the surface sequesters it from further activation and may protect it from additional enzymatic modifications. Internalization requires endocytosis and is dependent upon a variety of mechanisms, some of which involve β -arrestin, dynamin, and G-protein receptor kinases.^{133–135} Once internalized, the ligand can dissociate from the receptor, which is then returned to the cell surface or transported to lysosomes where it is degraded proteolytically. The role of internalization on tolerance has not been fully established, but some investigators have suggested a paradoxical relationship between internalization and tolerance in which failure to internalize leads to tolerance.¹³⁶ However, this concept remains quite controversial since a number of drugs that do internalize the receptor also produce tolerance.

A variety of kinases have been implicated in the neuroadaptive changes to opioid signal transduction following continued exposure to the drug.¹³⁷ When activated, kinases and phosphatases can have extensive effects on receptor function. Phosphorylation of opioid receptors has been associated with desensitization, while other receptors, such as the NMDA receptor which counters opioid responses, are activated.¹³⁷

Opioid–neurotransmitter interactions and tolerance

A number of transmitters and their receptors have been implicated in countering the actions of opioids, including glutamate and NMDA receptors, cholecystinin (CCK), γ -aminobutyric acid (GABA), dopamine, and nitric oxide.^{97,100,131,138} Early studies suggested that CCK antagonists could block morphine tolerance, but subsequent studies have suggested that this simply reflects a potentiation of morphine responses in general without a direct effect on tolerance.^{139,140} However, there is a direct involvement of the NMDA receptors–nitric oxide cascade in morphine tolerance, as noted earlier.^{96–99,101,141} Blockade of NMDA receptors or nitric oxide synthase can prevent or reverse pre-established opioid tolerance.

Other systems have also been implicated in tolerance. For example, blockade of delta opioid receptors with antagonists or antisense prevents the development of tolerance to morphine, as does disruption of the gene for the delta receptor or the enkephalins.^{142–144} Even more interesting were the observations that morphine treatment upregulates the expression of P-glycoprotein at the blood–brain barrier. P-glycoprotein plays a significant role in the blood–brain barrier and, as seen in knockout mice, its elimination enhances morphine analgesia. However, these same mice also display no tolerance to morphine.^{145–147} Thus, tolerance is an extremely complex collection of processes.

The complexity is even further increased since some of these systems can have opposing effects on opioid analgesia depending upon their location. For example, activation of NMDA systems within the rostral ventromedial medulla facilitates opioid analgesia by activating descending pain inhibitory tracts which dampen dorsal horn neurotransmission. Conversely, NMDA receptors within the dorsal horn are considered to be pronociceptive.^{148,149} GABA is antinociceptive within the dorsal horn, but within the peri-aqueductal grey it counters analgesia by inhibiting ‘off cells’, which are responsible for the descending inhibition of dorsal horn sensory processing. GABA-A receptor agonists, such as muscimol, produce hyperalgesia and hypersensitivity in the peri-aqueductal grey.^{150–153} Finally, some splice variants of neuronal nitric oxide are involved with tolerance and its decrease in morphine sensitivity while other splice variants enhance morphine actions.^{154–156}

Opioid-induced hyperexcitability

Occasional patients receiving spinal opioids over a prolonged period of time develop hyperaesthesia with high doses of morphine.^{157–159} This paradoxical pain is most commonly associated with high doses of morphine.¹⁶⁰ These abnormal pain states initiated by opioids resemble neuropathic pain qualitatively and differ from the original pain for which the opioid was initiated. However, these pain states are not common and clinical importance remains a matter of debate. It has been suggested that opioid-induced pain, neuropathic pain, and opioid analgesic tolerance share some of the same underlying neurophysiological mechanisms.^{57,159,161,162} It is believed that this hyperalgesia may be related to the hyperexcitability seen in cells with low doses of morphine,^{19,120} but this is uncertain in view of the need for high, rather than low, spinal morphine doses to induce the syndrome. More commonly, it is mediated by metabolic factors, such as the accumulation of morphine-3-glucuronide, which is a toxic metabolite with no affinity for opioid receptors, or general metabolic imbalances. In some situations, it comes at the end of the opioid

dose, suggesting that it may be associated with the early signs of withdrawal. In this case, administering the drug more frequently can alleviate the problem.

Opioid dosing

The optimal dosing schedule for opioids is still unsettled. Many clinicians have long recommended 'around the clock' dosing, in which the opioids are given at fixed intervals without waiting for the pain to reappear. This has many advantages, primarily in the fact that the patient does not need to suffer while waiting for their medication to act. Some investigators have suggested that intermittent opioids for chronic pain lead to 'mini-withdrawal' responses which interfere with opioid actions.¹⁶³ Persistent hyperalgesia also has been reported long after opioid withdrawal.^{164–166} It has been suggested that both opioid withdrawal and naloxone increase spinal glutamate release and NMDA receptor activation, and may lead to more analgesic tolerance.¹⁶⁷ On the other hand, very low doses of naloxone sufficient to selectively block the excitatory effects of the drug are pro-analgesic. Thus, this remains a complex area of investigation with uncertain clinical implications. However, the advantages of maintaining the patient pain free along with the studies showing a decreased overall analgesic requirement with patient-controlled analgesia strongly support the concept of 'around the clock' dosing. Clinicians should be careful and be aware of the durations of the drugs they are using, particularly the long-lasting agents which may take several days to reach steady state levels, and yet not dose patients at intervals which allow a period of withdrawal. This is particularly problematic with methadone, and care should be taken to avoid too rapid escalation in dose, keeping in mind that it takes three to five half-lives to achieve equilibrium. This may mean 3–5 days for methadone and some of the long-acting formulations.

Mu opioid receptor genetics

All three opioid receptor classes have been cloned and all are members of the G-protein coupled receptor family, with their traditional seven transmembrane domains.^{78,105,118,168,169} The first coding exon of each encodes the N-terminus and the first transmembrane domain, while the second coding exon encodes the next three transmembrane domains, and the third coding exon encodes the last three transmembrane domains and the C-terminus (Fig. 1.6). The only exception is MOR-1, which has an additional fourth coding exon at the 3-end responsible for the last 12 amino acids in the intracellular C-terminus.^{118,170–173} The three opioid receptors share 60% amino acid

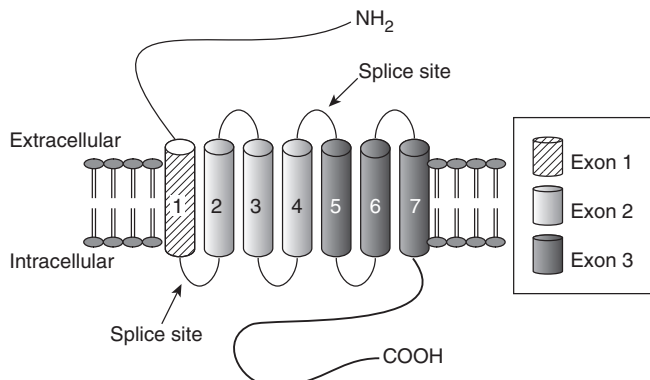


Fig. 1.6 Schematic structure of the DOR-1 and KOR-1 opioid receptors.

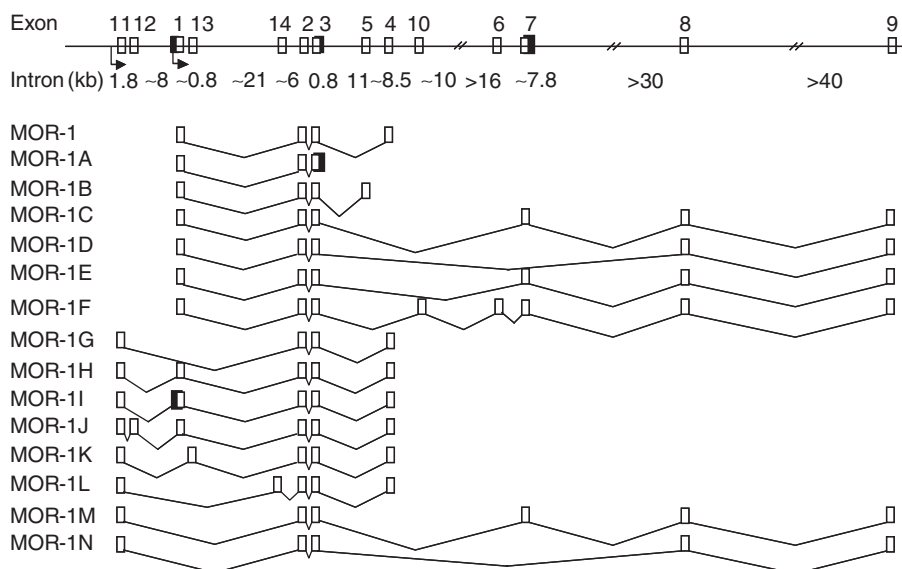
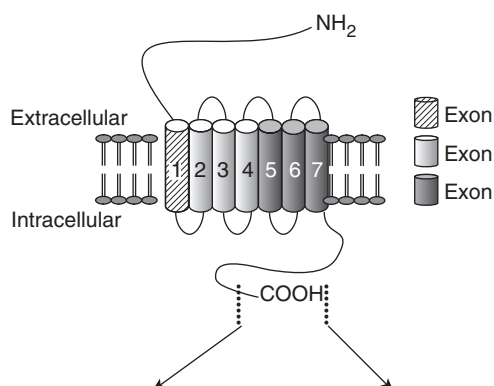


Fig. 1.7 Schematic of the alternative splicing in the mouse MOR-1 gene.

identity, predominantly in the transmembrane domains, and receptor homology among species is very high, particularly the second, third, and seventh transmembrane domains.¹⁷⁴ The second and third intracellular loops are also well conserved among the three opioid receptors and are the principle sites for G-protein coupling.¹¹⁸ The C-terminus tail is not well conserved among the major opioid receptors and has a number of putative phosphorylation sites.

Alternative splicing

Only one gene encoding a mu receptor has been identified, leaving open the question of its relationship to the mu receptor subtypes implied from the pharmacological studies. Soon after the initial cloning, a splice variant was identified in a human cell line⁵² and rat brain.⁵⁷ Subsequently, a host of splice variants were identified in mice (Fig. 1.7), with similar splicing patterns in rats and humans.^{53-56,175} The most common pattern among the species involves splicing at the C-terminus (Fig. 1.8). This splicing is downstream from exon 3 and involves replacement of exon 4 with a series of alternative exons. This is interesting, since both the kappa receptor KOR-1 and the delta receptor DOR-1 only have three coding exons and do not have an analogous exon 4. These C-terminus MOR-1 splice variants all contain identical transmembrane domains which are thought to comprise the binding pocket, but differ at the tip of their C-terminus (Fig. 1.8). Thus, it is not surprising that they all show similar affinities and selectivities for mu ligands (Table 1.4). Despite these similarities in binding affinities, the mu opioids can vary enormously in terms of their relative efficacies and potencies at these variants (Fig. 1.9), presumably because of the differences in the C-terminus. For example, the potencies of fentanyl and DAMGO were quite similar for all the variants tested, while dynorphin A and β -endorphin varied markedly. The efficacy of the drugs also varied from variant to variant (Fig. 1.9B). Although methadone showed similar efficacies, as defined by the maximal stimulation of [³⁵S]GTP γ S binding, β -endorphin showed a wide range. However, what makes these observations most interesting was the difference in rank order of the drugs from one variant to another. β -Endorphin was more efficacious than fentanyl in MOR-1E, but fentanyl was more efficacious in MOR-1. Thus, the ability of the various mu



	<u>Amino Acid Sequence</u>	<u>Exons</u>
MOR-1	LENLEAETAPLP	4
MOR-1A	VRSL	
MOR-1B	KIDLF	5
MOR-1C	PTLAVSVAQIFTGYSPTHV EKPKSCMDRGMRNLLPD DGPRQESGEGQLGR	7,8,9
MOR-1D	RNEEPSS	8,9
MOR-1E	KKKLDQRGCVQHPV	6,7,8,9
MOR-1F	APCACVPGANRGQTKASDL LDLELETVGSHQADAETNP GPYEGSKCAEPLAISLVPLY	10,6,7,8,9

Fig. 1.8 Schematic of the C-terminus splicing of mouse MOR-1 variants.

Table 1.4 Affinity of opioids for MOR-1 variants

Ligand	K_i value (nM)					
	MOR-1	MOR-1A	MOR-1C	MOR-1D	MOR-1E	MOR-1F
Morphine	5.3 ± 2.5	3.1 ± 0.5	2.7 ± 0.8	1.6 ± 0.2	2.4 ± 0.6	3.0 ± 0.6
M6G	6.4 ± 2.4	5.0 ± 1.5	4.5 ± 1.8	4.8 ± 0.9	5.6 ± 0.9	9.6 ± 1.0
Methadone	1.4 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	1.4 ± 0.1	0.7 ± 0.3	1.3 ± 0.2
Fentanyl	2.3 ± 1.0	1.5 ± 0.6	1.2 ± 0.4	3.3 ± 1.5	1.2 ± 0.5	1.7 ± 0.5
DAMGO	1.7 ± 0.4	1.0 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	1.1 ± 0.3
Dynorphin A	10.5 ± 0.7	8.2 ± 2.8	4.6 ± 1.1	2.7 ± 0.8	8.9 ± 1.1	12.1 ± 1.0
α-Endorphin	8.4 ± 4.9	4.3 ± 1.0	5.8 ± 0.5	1.7 ± 0.5	4.9 ± 1.2	6.0 ± 1.6
[Met] ⁵ enkephalin-Arg ⁶ -Phe ⁷	4.1 ± 1.0	3.5 ± 1.3	2.1 ± 0.7	3.7 ± 1.2	4.4 ± 0.9	3.9 ± 0.7
Endomorphin 1	2.1 ± 0.9	2.3 ± 0.3	1.1 ± 0.3	1.8 ± 0.3	2.3 ± 0.2	2.9 ± 1.1
Endomorphin 2	4.2 ± 2.3	3.9 ± 0.8	1.5 ± 0.2	2.0 ± 0.4	4.4 ± 1.0	4.1 ± 1.6
U50,488H	>500	>500	>500	>500	>500	>500
DPDPE	>500	>500	>500	>500	>500	>500

Receptor competition binding studies were performed on CHO cells stably transfected with the indicated splice variant using the mu agonist ³H-DAMGO. Results are from the literature.¹⁷⁷

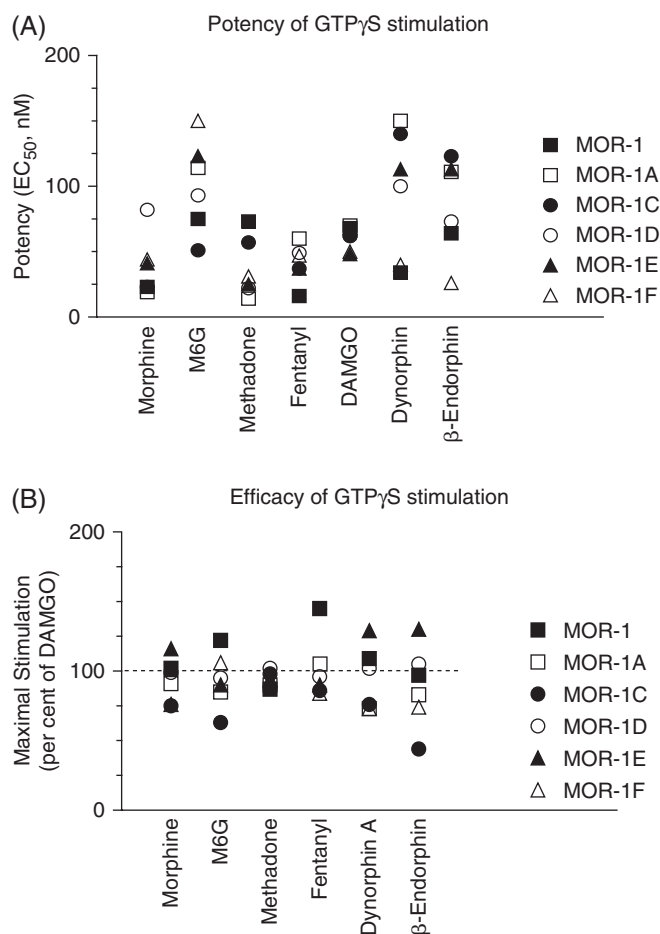


Fig. 1.9 Activation of the MOR-1 variants. The ability of the indicated drugs to activate the receptors expressed in CHO cells was assessed by their ability to induce the binding of a stable GTP analog, [35 S]GTP γ S. (A) The potency of the drugs was assessed by their ED₅₀ values. (B) Efficacy was determined by the maximal stimulation of the drug in each variant and expressed as a percentage of the maximal effect of DAMGO. From the literature.¹⁷⁷

opioids to activate these receptors varies markedly among the variants and among each other. These functional differences may help explain the variability among the drugs seen clinically.

Within the brain, the variants have distinct regional distributions.^{176–179} Even in areas which contain more than one variant, there is evidence for the expression of the variants in different cells. Thus, these variants display region- and cell-specific processing. They also differ ultrastructurally; MOR-1 is localized to both pre- and postsynaptic regions, whereas MOR-1C is almost exclusively localized presynaptically. Additional studies illustrate that the variants are also associated with different neurotransmitters. Whereas MOR-1C is associated with neurons containing CGRP at both the light and ultrastructural level, MOR-1 is not.¹⁷⁹

Recent work has also uncovered splicing at the N-terminus, with exon 11 and its own independent promoter located greater than 20 kb upstream from exon 1.^{53,180} While some of the variants generated by the exon 11 promoter encode truncated proteins, evidence is accumulating

that they may be pharmacologically important. In addition, three of these exon 11 variants also encode the same protein as MOR-1 itself, leading to the question of why four different splice variants under the control of two different promoters generate an identical protein.

Dimerization of opioid receptors

Receptor heterogeneity can also be achieved through receptor dimerization.^{118,181–183} Both homo- and heterodimerization have been observed. Dimerization is common among G-protein receptors and has been reported to involve interactions extracellularly, intracellularly, and between the transmembrane domains, depending upon the receptor. With the opioid receptors, it has been suggested that dimerization requires interactions between transmembrane domain 5 in one receptor and transmembrane domain 6 in the other to form an interface between the two receptors within the lipid layer.¹⁸⁴

Opioid receptor dimerization can alter opioid receptor selectivity and trafficking.¹⁸⁵ Heterodimers may have different opioid binding profiles compared with monomers, as shown by the association of DOR-1 and KOR-1^{185,186} to form a receptor consistent with the kappa₂ receptors first proposed from binding assays.⁵⁸ Perhaps the most prominent change in ligand selectivity within the opioid field is the dimerization of MOR-1 and the orphanin FQ receptor, ORL-1 (also known as KOR-3).¹⁷⁴ Orphanin FQ/nociceptin (OFQ/N) binds to its own receptor with very high affinity and is insensitive to traditional opioids. Co-expression of the ORL-1 (KOR-3) receptor with MOR-1 changes this selectivity, with standard opioids competing with OFQ/N binding quite effectively.

Clinical relevance

The cardinal principle of pain management is the need to individualize therapy. Clinical observations have long documented a wide range of responses among individuals to different mu opioids, actions that can be recapitulated in animal models. Unfortunately, the choice of drug for an individual patient remains empirical. There is no way to anticipate which one will be optimal. Thus, the clinician is faced with the need to switch therapies until an effective one is found. Pure opioid agonists have no ceiling effect on pain control, and so escalation of drug dose can enhance responses. However, dose escalation is often limited by side effects. With chronic dosing, all patients will become both tolerant and physically dependent. When the dose of the drug can no longer be increased, it is common to switch the patient to an alternative opioid, a concept termed 'opioid rotation'.¹⁰³ By changing the drug, it is often possible to restore analgesic effectiveness because of the presence of incomplete cross-tolerance. However, it is important to note that the relative potency of opioids changes in tolerant patients, and the equivalent ratios commonly published for opioid-naïve patients cannot be used. Indeed, when switching drugs in a highly tolerant patient, it is common practice to reduce the anticipated dose of the second drug by 50% or even more to avoid overdosing the patient.

Despite the known benefits of opioids in controlling pain, they are rarely used alone in pain management. A wide range of adjuvant drugs are available and effective. These range from NSAIDs to antidepressants. NMDA antagonists also have the theoretical advantage of reducing tolerance, although this has not yet been demonstrated clinically. The wide range of responses among patients to adjuvant analgesics requires individualization of therapy.

Conclusions

Opioid pharmacodynamics are both unique and complex. Advances in molecular medicine have unravelled many of the mysteries behind the wide diversity of opioid responses among individuals,

but many more remain. Our present understanding of opioid receptor genetics and its molecular pharmacology opens new avenues in the design and development of new agents. Equally important, it provides a scientific foundation to support what clinicians have known for centuries. All patients and their pain are unique, and so must be their treatments.

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