Opioids and Opiates: Pharmacology, Abuse, and Addiction

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Abstract

This chapter presents an overview of the opioid system consisting of a wide variety of endogenous and exogenous ligands, as well as several receptor types, which activate complex intracellular signaling pathways. The chapter begins with a brief history of opiate use, opioid receptor discovery, and the identification of main mechanisms of action. It also describes the effects and pharmacological

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properties of main opioid receptor ligands (agonists, partial agonists, antagonists, agonist-antagonists, and inverse agonists), emphasizing clinically relevant compounds. The structure of opioid receptors is reviewed along with a description of receptor types and putative receptor subtypes, G-protein receptor signaling, opioid receptor regulation, and β-arrestin-dependent signaling. Furthermore, there is a section concerning splice variants, single-nucleotide polymorphisms of the μ-opioid receptor, and receptor dimerization. Other mechanisms that modulate ligand–opioid receptor complexes are presented, such as biased agonism, the action of positive and negative allosteric modulators, and epigenetic factors. The final sections are dedicated to the definition and neurobiology of opioid misuse, dependence and tolerance, and an overlook of some of the main challenges posed by opioids as medications and misused drugs.

Keywords

Opioids • Absorption and distribution • Addiction • Physical dependence and tolerance • Agonist–antagonists • Agonists • Effects • History • Inverse agonists • Metabolism and excretion • Receptor ligands • Receptors

Brief History

Opium (from a Greek word that means "juice") is the milky exudate of unripe seed capsules of the poppy plant *Papaver somniferum*. There is evidence that ancient civilizations in Mesopotamia and Egypt were familiar with opium's effects. The Ebers Papyrus describes a mixture of opium and other compounds that was used for quieting crying children in Egypt. The first undisputed mention to poppy juice is attributed to the Greek philosopher Theophrastus in the third century BC. In the medieval period, Avicenna of Persia described opium as the most powerful of the stupefacients in his "Canon of Medicine." Avicenna not only mentioned the hypnotic, antitussive, and gastrointestinal effects of opium; he also recognized its potentially poisonous effects. In the seventeenth century, Thomas Sydenham promoted the use of an opium product known as "laudanum" (from the Latin word *laudare*, meaning "to praise") for treating dysentery and other conditions. Laudanum became very popular in different cultures and was considered a panacea; however, opium preparations were variable in composition, and while some batches were clearly effective, others produced excessive narcosis or no effect.

In 1803, the German pharmacist Friedrich W. Sertürner isolated the active compound of opium and called it *morphium*, after Morpheus, the Greek god of dreams. A few years later, Gay-Lussac changed its name to *morphine*. Codeine, another naturally occurring opium alkaloid, was isolated in 1832, but it did not come into clinical use until 1880. Heroin (diacetylmorphine) was synthetized from morphine as a more potent analgesic and antitussive and commercialized by the end of the nineteenth century. Physicians were not aware of the high addiction liability of heroin until some years later, when the number of subjects dependent to heroin was

too high to be ignored. In an attempt to limit opiate use to medical purposes, in 1914 the Harrison Narcotics Act was passed in the United States requiring physicians to report their opiate prescriptions.

Although isolated from opium in the early 1800s, the structure of morphine was not identified until 1925. In the following decades, the search for effective analgesics with less negative side effects led to the active synthesis and screening of many chemically related drugs. It was in 1973 when Candace Pert and Solomon Snyder demonstrated the existence of stereospecific opiate binding sites in the nervous system. In the following years, endogenous opioids and their precursor were identified. The variety of ligands with different structures and pharmacological profiles in diverse experimental preparations led to the proposal of the existence of receptor subtypes by 1967, but the identification of opioid receptors as biochemical entities took place in the early years of the 1990s (Booth 1997; Snyder and Pasternak 2012). The last decade has seen a dramatic increase in the research related to the sites and mechanisms of action of opioids, along with an epidemic use of prescription drugs and overdose deaths. Some milestones in opioid history are summarized in Table 1. Today, as throughout history, the use of opiates for human well-being with little or no negative effects remains a significant challenge. This chapter aims to present a brief review of the main ligands, receptors, and effectors involved in acute and chronic opioid effects.

Terminology

The term *opiate* was first used to refer to substances found in the poppy plant but was later expanded to include compounds directly derived or synthesized from thebaine (a natural opium alkaloid) and its derivatives, with or without synthetic modifications. In this sense, morphine, heroin, codeine, and naloxone are correctly considered opiates, but completely synthetic compounds such as fentanyl are not. *Opioid* is a broader term that includes endogenous or exogenous substances that bind to one or more types of opioid receptors. *Endogenous opioids* are naturally occurring ligands for opioid receptors. Opiates are also referred to as narcotic analgesics, but the term narcotic (a drug that causes sleep) is frequently used as synonym with abused drug, and it will not be used here despite its historical relevance (Kreek 2007).

Opioid Effects

Effects on the Nervous System

Morphine and its derivatives relieve pain by activating opioid receptors, particularly the μ-opioid receptor. Analgesia, euphoria, respiratory depression, cough suppression, pupillary constriction, nausea, and vomiting are the most prominent central nervous system (CNS) effects of opioids. Analgesia is produced through inhibition

Table 1 Timeline of opioid history and research

Date	Events
c. 3000 BC	Opium poppy is used and cultivated in ancient cultures
c. 1300 BC	There is evidence of poppy fields and opium trade in Egypt
1020	Avicenna teaches that opium is "the most powerful of stupefacients"
1680	Thomas Sydenham introduces laudanum, a compound of opium, wine, and herbs, as a remedy for numerous ailments. The drink rapidly becomes very popular
1803	Friedrich Sertürner isolates the active compound of opium and names it "morphium" (morphine)
1827	E. Merck and Co. begins commercial manufacturing of morphine
1839–1841	The British send warships to China in response to China's decision to suppress the opium traffic. The First Opium War begins. In 1841, China is forced to pay an indemnity and to cede Hong Kong to Britain
1843	The hypodermic syringe is introduced and, with it, a new and more efficient route of morphine administration
1856	The Second Opium War. China is defeated and forced to legalize opium importation
1898	The Bayer Company introduces heroin (diacetylmorphine) for medical use
1903	Heroin addiction rises to alarming rates
1914	The Harrison Narcotics Act is passed. Opium can be sold only with prescription
1925	Morphine's chemical structure is identified
1950s–1960s	Clinical and preclinical characterization of different opiate compounds leads to proposals and models of opioid receptors. In 1967, Billy Martin suggests the existence of more than one opiate receptor
1972	Methadone, first synthetized for use as analgesic in the Second World War, is approved by the Food and Drug Administration (FDA) for use in treating opiate addiction
1973	Opioid receptors are identified and characterized in binding assays
1975	Identification of endogenous opioids
1976–1981	Demonstration of mu, delta, and kappa opioid receptors
1992–1993	Cloning of delta, mu, and kappa opioid receptors
1994	Cloning of the nociceptin/orphanin FQ receptor
2002	The FDA approves buprenorphine products for use in opiate addiction treatment
2010s	Extensive characterization of biased ligands, allosteric modulators of opioid receptors, single-nucleotide polymorphisms (SNPs), and opioid receptor dimers

of ascending pathways carrying pain information gathered from primary sensory neurons and activation of descending pain control systems through the rostral ventromedial medulla down into the dorsal horn of the spinal cord. Opioids produce respiratory depression by reducing the brain stem respiratory centers' responsiveness to increased carbon dioxide levels. Respiratory arrest is nearly always the cause of death produced by heroin and prescription drug overdose. Cough reflex is inhibited by a direct neurodepressant effect on the medulla. Nausea and vomiting are produced by activation of the chemoreceptor trigger zone for emesis in the medulla. Constriction of the pupil (miosis) is due to stimulation of the parasympathetic nerve

innervating the pupil. This is an indirect effect of opioids through the inhibition of GABAergic interneurons that regulate the parasympathetic outflow. With high doses of agonists, miosis is marked (pinpoint pupils) and is considered a pathognomonic sign of opioid intoxication.

Opioids mildly reduce peripheral resistance and blood pressure. Intravenous administration of different opioid agonists increases the incidence of these adverse effects. The risk of severe orthostatic hypotension is higher in individuals who have lost blood.

Opioids are useful antidiarrheal drugs because they delay gastric emptying, slow intestinal transit time, and produce spam of the anal sphincter. Opioid receptor agonists also induce an increase in biliary tract pressure that may result in biliary spasm or colic, especially in the sphincter of Oddi.

Several opioids release histamine, which may cause facial itching, sweating, flushing, and warmth of the face, neck, and upper thorax. The actions of opioids on neuroendocrine function are complex, but in general, they can decrease the level of hormones under the control of the hypothalamus-pituitary-adrenal axis, including sex hormones. Kappa agonists inhibit the release of antidiuretic hormone causing diuresis (Trescot et al. 2008).

Opioids are invaluable agents for the relief of pain. They can be indicated for preoperative medication and support of anesthesia and as adjunctive therapy in patients with dyspnea associated with pulmonary edema secondary to acute left ventricular dysfunction. Adverse acute effects include dizziness, constipation, weakness, and mental clouding, among others. Respiratory depression is the main hazard associated with opiate use. Also, repeated use can lead to tolerance, physical dependence, and addiction (Inturrisi 2002).

Effects on the Immune System

A complex interaction exists between the nervous and the immune system. Relatively recent studies have shown that morphine, particularly in high doses, may be immunosuppressive. This could be due to direct and indirect actions. Opioids have immunomodulatory effects acting on both classical naloxone-sensitive opioid receptors and non-naloxone-sensitive receptors expressed in cells involved in host defense and immunity. Opioid receptors have been described in practically all types of immune cells, including B and T lymphocytes, macrophages, granulocytes, and monocytes. These receptors have similar biochemical and pharmacological characteristics and are encoded by the same genes as neuronal opioid receptors. Moreover, activation of these receptors triggers the same signaling pathways as in neuronal cells and modulates immune cell proliferation, chemotaxis cytotoxicity, cytokine and chemokine receptor expression, cytokine synthesis, and secretion in vitro (Sharp 2006).

One of the most important actions described for opiates is that they have anti-inflammatory properties. These “non-analgesic” effects of opiates have been described in a number of different peripheral immune cells that control the release

of cytokines and chemokines responsible for inflammation. The targets of opioids on immune cells include transcription factors and specific kinases, as well as modulation of cell functions, such as intracellular viral replication, calcium release from intracellular stores, cAMP synthesis, respiratory burst activity, and other effector responses. There are relatively few studies comparing the effects of chronic opioid administration on the immunological system, but some evidence suggests that significant variations among μ-opioid receptor agonists exist (Molina-Martínez et al. 2014).

Opiate effects have been reported in mice lacking classical opioid receptors (triple knockout mice). In those animals, opiates produce hyperalgesia. Microglia cells play an important role in inflammatory responses that contribute to reduce analgesia and seem to participate in tolerance development. For these reasons, several researchers have studied the effects of opioids on microglial cells, particularly on TLR4 receptors. TLR4 is a toll-like receptor activated by lipopolysaccharide from Gram-negative bacteria. In vitro and in silico results strongly suggest that opioids interact directly with the TLR4 receptor complex, particularly with its associated protein MD-2, modulating cytokine production in a classical opioid receptor-independent fashion (Hutchinson et al. 2011). The consequences of this interaction on cytokine production or macrophage effector functions have not been fully addressed, but are of interest due to the association of chronic opiate use and increased susceptibility to infections.

Opioid Receptor Ligands

Endogenous Opioid Peptides

Opioid analgesics activate an endogenous modulating system comprised of endogenous opioid peptides and their receptors. Several distinct families of endogenous opioid peptides have been identified: enkephalins, endorphins, dynorphins, and endomorphins (Table 2). In addition, nociceptin is an endogenous-related peptide that binds to the nonclassical nociceptin opioid peptide (NOP) receptor.

The enkephalins (meaning “in the brain”) were the first natural ligands for opioid receptors identified in pig brains’ extracts. They are two pentapeptides differing only in the last amino acid: met-enkephalin (Tyr-Gly-Gly-Phe-Met) and leu-enkephalin (Tyr-Gly-Gly-Phe-Leu). Enkephalins are widely distributed in the body with high concentrations in the brain, gastrointestinal tract, and the adrenal medulla. They bind to both μ and δ receptors and are derived from the larger precursor proenkephalin. Proenkephalin contains multiple copies of met-enkephalin and a single copy of leu-enkephalin.

Table 2 Amino acid sequence of some endogenous opioid peptides

Peptide	Amino acid sequence
[Leu]-enkephalin	**Tyr-Gly-Gly-Phe-Leu**
[Met]-enkephalin	**Tyr-Gly-Gly-Phe-Met**
Endomorphin-1	**Tyr**-Pro-Trp-**Phe**
Endomorphin-2	**Tyr**-Pro-Phe-**Phe**
Heptapeptide	**Tyr-Gly-Gly-Phe-Met**-Arg-Phe
α-Neoendorphin	**Tyr-Gly-Gly-Phe-Leu**-Arg-Lys-Tyr-Pro-Lys
Dynorphin B	**Tyr-Gly-Gly-Phe-Leu**-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr
Dynorphin A	**Tyr-Gly-Gly-Phe-Leu**-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln
Nociceptin	**Phe-Gly-Gly-Phe**-Thr-Gly-Ala-Arg-Lys-Se-Ala-Arg-Lys-Leu-Ala-Asn-Gln
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

Pro-opiomelanocortin (POMC) is the precursor protein not only of the potent opioid peptide β-endorphin but also of several non-opioid peptides including α-melanocyte-stimulating hormone (α-MSH), adrenocorticotropic hormone (ACTH), and β-lipotropin (β-LPH). POMC is found in high concentrations in the pituitary gland, which releases a variety of hormones in response to releasing factors. In particular, stress produces corticotropin-releasing factor (CRH) in the hypothalamus, and CRH causes an increase in ACTH and β-endorphin production in the anterior pituitary. This effect has been associated with stress-induced analgesia.

Prodynorphin, the precursor of dynorphins, has three leu-enkephalin core opioid sequences, and differential processing can lead to various opioid peptides. Prodynorphin products, dynorphin A, dynorphin B, and neoendorphin, are abundant in neurons throughout the brain and spinal cord.

Nociceptin, usually referred to as nociceptin/orphanin NQ, is a neuropeptide of 17 amino acids derived from its precursor pronociceptin. Nociceptin is involved in the transmission of pain and other functions, including the neuroendocrine stress response (Kreek 2007).

Endomorphins are tetrapeptides with analgesic effect, high affinity, and selectivity for the μ-opioid receptor. The mechanism for the endogenous synthesis of endomorphins has not yet been identified.

Endogenous peptide precursors are widely distributed in the CNS. They are synthesized in the nucleus and transported to the nerve terminal where they are cleaved by specific processing enzymes that recognize double basic amino acid sequences positioned before and after the opioid peptide. Each of the opioid

precursors contains multiple active peptides, which can be modified differentially in different brain areas. They also often coexist with other neurotransmitters or neuropeptides. Processing of these peptides is altered by physiological demands, and the final product produced by and stored within a given neuron depends not only on the precursor, but also on the enzymes available to process the precursor in certain ways. Endogenous opioid peptides play a regulatory role in the response of the organism to physiological and environmental demands. They are involved in the response to pain, regulation of the hypothalamic-pituitary-adrenal axis, and other neuroendocrine functions critical for survival.

Regarding the affinity for receptor subtypes, met-enkephalin, leu-enkephalin, and β-endorphin bind to both μ and δ receptors; endomorphins are selective for μ receptors and dynorphin A for κ receptors. The amino acid sequences of main endogenous opioid peptides are shown in Table 2.

Morphine and Its Derivatives

Chemical Classes

The structure of morphine and some derivatives are shown in Fig. 1. Distinctive features of morphine include a phenanthrene skeleton (three fused benzene rings; in blue), a furan ring (composed of one oxygen and four carbon atoms; in green), and a piperidine ring with an *N*-methyl group and a quaternary carbon (in red). In addition, two hydroxyl groups are attached to carbons at positions 3 and 6 (C3 and C6). Several natural and semisynthetic compounds have similar structures with substituents in the nitrogen atom, one or both hydroxyl groups. Replacing the *N*-methyl with larger radical groups such as allyl, cyclopropyl, or cyclobutyl results in compounds with opioid-receptor antagonist properties. This is the reason why the names of several antagonists begin with *Nal-*, like nalorphine (*N*-allyl morphine), the first opioid-receptor antagonist synthesized. Other morphine derivatives have in common that they have oxygen in C6, a single bond between C7 and C8, and a hydroxyl group added to C14 (Fig. 1). Because of the oxygen in C6, their names finish with *–one* (like ketone: −C=O). Some examples of these drugs are oxymorphone, oxycodone, naloxone, and naltrexone. The lack of the furan ring gives rise to other group of synthetic compounds known as morphinans, of which levorphanol, a μ-opioid receptor agonist with longer duration of action than morphine, is an example (Fig. 1). The D-isomer of levorphanol, dextrorphan, is not active as an analgesic, but has inhibitory effects at NMDA receptors.

Benzomorphan analogs are compounds with a three-ring simplified structure (Fig. 2). Some examples are ketocyclazocine, pentazocine, cyclazocine, and bremazocine. These agents differ in receptor selectivity (they have high affinity for

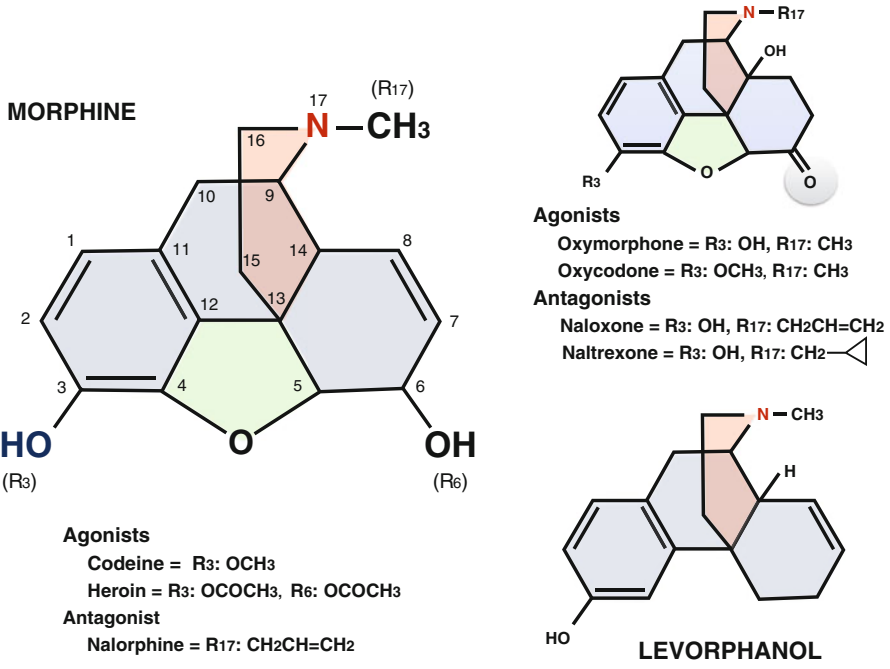

Fig. 1 Chemical structure of morphine and selected morphine-like drugs. The phenanthrene skeleton is depicted in *blue*, the furan ring in *green*, and the piperidine ring in *red*

κ receptors) and in their ability to produce analgesia without causing some of the side effects common to morphine and other classical opioids. Phenylpiperidine drugs have a phenolic group and the piperidine ring as common structural components. Examples of this group are the synthetic and potent derivatives meperidine (also known as pethidine) and fentanyl. Other compounds such as etorphine and buprenorphine have six rings instead of five. Etorphine is several thousand times more potent than morphine and is used for immobilizing large animals. Buprenorphine has long-lasting effects and is used for facilitating recovery from opiate use disorders. Methadone and dextropropoxyphene pertain to a group of drugs that bear little apparent chemical resemblance to morphine, but have the basic structural features necessary to activate opiate receptors (a phenolic ring, a protonated hydrogen, and a hydrophobic domain) and produce similar pharmacological and behavioral effects (Trescot et al. 2008). The structures of some of these agents are shown in Fig. 2.

Intensive research and active chemical synthesis have produced a wide variety of opioid receptor ligands with differences in receptor selectivity, pharmacokinetic

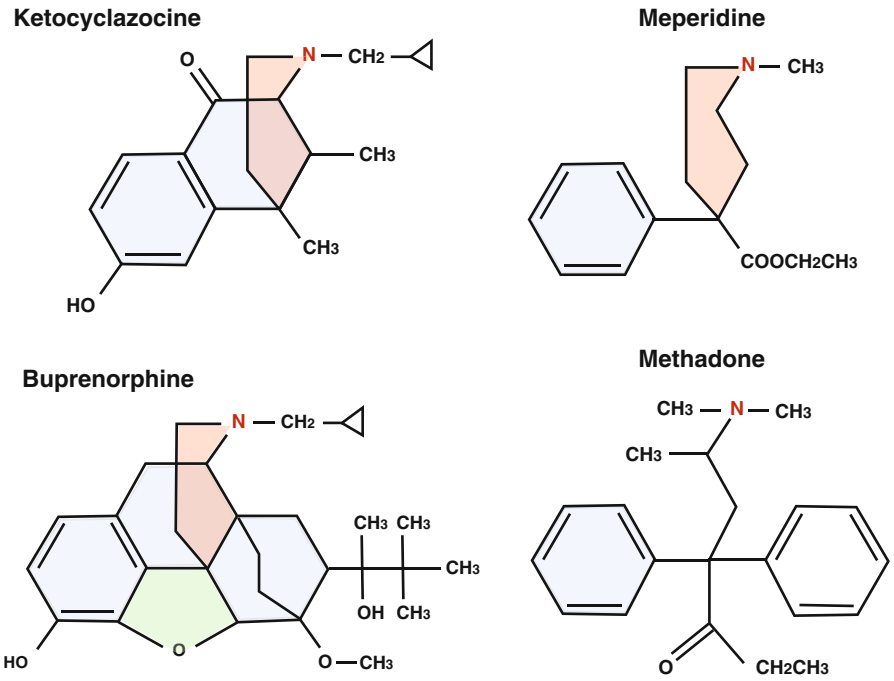

Fig. 2 Chemical structure of ketocyclazocine, meperidine, buprenorphine, and methadone

parameters (absorption, distribution, duration of effects, metabolism, etc.), and pharmacological effects (potency, efficacy, agonist, or antagonist actions). Some characteristics of the most relevant compounds are summarized in the following sections.

Agonists, Antagonists, Agonist-Antagonists, and Inverse Agonists

Agonists bind to physiological receptors mimicking the effects of the endogenous opioid peptides. A drug that binds to the same site than the endogenous agonists is an orthosteric agonist; if it binds to a different region, it is an allosteric agonist. Antagonists are compounds that block, reduce, or counteract the action of an agonist. They can do it by direct competition with the agonist for the same site on the receptor (competitive antagonists) or by indirect or functional antagonism. Partial agonists produce lower responses than full agonists even at doses high enough to occupy all of the receptors available in a tissue. Many G-protein-coupled receptors (GPCRs) show varying degrees of basal or constitutive activity, which means that they can activate G

proteins even when not occupied by agonists. This constitutive activity is usually minimal, but can be increased under specific circumstances. Interestingly, when certain ligands bind these constitutively active receptors (R*), they produce effects that are opposite to pure agonists. Such ligands are called inverse agonists, and their effects can be observed only when constitutive activity is evident (Burdford et al. 2015). It is worth noting that partial agonists and inverse agonists that interact with a full agonist will behave as competitive antagonists. Finally, due to the existence of several receptor subtypes, some drugs can produce an agonist effect at one receptor and an antagonist effect at another; they are called mixed agonist-antagonists.

Agonists may differ in affinity, efficacy, and potency. The affinity of opioid receptor ligands is usually very high in the nanomolar or subnanomolar range. The *potency* of a drug is the amount of drug needed to produce a determined effect and can be estimated by the EC_{50} (concentration needed to produce 50 % of the maximal response) from a concentration-response curve. Fentanyl, for instance, is more potent than morphine because it produces analgesia with a hundredth of morphine's active dose. Potency is a function of the affinity and efficacy of a compound. A potent drug binds and activates receptor–effector complexes at low concentrations.

Efficacy refers to the ability of a drug to produce a cellular response after binding to the receptor. Low-efficacy agonists need to occupy a larger fraction of the available receptors to produce their effects than agonists with high efficacy. This property cannot be directly determined from the maximal response obtained in a dose–response curve because efficacy is dependent not only on the drug (*intrinsic efficacy*) but also on the tissue, and a full agonist in one tissue might not be a full agonist in another. Moreover, the efficacy of a substance might be difficult to establish due to specific experimental limitations (e.g., when a cutoff value is established to prevent tissue damage in experiments where the response to a noxious stimulus is evaluated). Among the variables related to the tissue that can affect the efficacy of a drug, we can mention the presence of receptor subtypes, how many of these receptors are in a constitutively active state, if they are desensitized, internalized, etc., as well as the quantity and type of specific enzymes and ion channels available. A related concept is *intrinsic activity*, defined as the maximal response that a substance can produce in a specific tissue. It is a relative value with respect to the maximal response achievable by a drug considered as a full agonist with an intrinsic activity of one (Kelly 2013).

In summary, in order to determine whether a ligand behaves as a full agonist or a partial agonist, it is necessary to consider various factors such as the affinity for specific receptor subtypes, the transduction system under investigation, the history of previous opiate exposure, and the tissue/experimental preparation where the drug is tested. As to antagonists, several drugs block the effects of opioid receptor agonists most of the time, but can behave as inverse agonists when there is an overexpression of R*. Examples of this are naloxone, naltrexone, and ICI 174864.

Clinically Relevant Opioids

Agonists

Morphine-like agonists share with morphine desirable and undesirable pharmacologic effects; therefore, all of them are analgesics and can produce some or all of the adverse effects associated with μ-opioid receptor stimulation. Despite this, different drugs vary in aspects that need to be considered when it comes to choosing a specific analgesic, including their pharmacokinetic properties (time to peak effect, duration of actions, metabolism, etc.), relative potency, oral to parenteral efficacy ratio, and drug–drug interactions. Table 3 summarizes some characteristics of clinically relevant opioids.

Heroin (diacetylmorphine) is more potent and crosses the blood–brain barrier more easily than morphine. Consequently, heroin achieves higher concentrations in the CNS when it is smoked or i.v. injected, producing an intense high. In some countries like the United Kingdom, heroin can be used clinically, but it is mainly misused and sold illegally throughout the world. The risk of adverse effects associated with heroin use is elevated due to its high potency and efficacy.

Codeine and *hydrocodone* are compounds commonly prescribed to suppress cough at doses lower than those needed to produce analgesia. Both agents are also used for the relief of mild to moderate pain and are marketed alone or combined with acetaminophen or aspirin. Because they are μ−opioid receptor agonists, they can produce euphoria, respiratory depression, and dependence. When a tablet containing hydrocodone or codeine is crushed, dissolved, and injected, it produces significant psychoactive effects and increases the risk of opioid overdose along with toxicity caused by non-opioid compounds.

Oxycodone is another morphine-like agent available in continuous-release preparations for the management of moderate to severe pain. *Hydromorphone*, a short-acting potent opioid drug, can be used as a substitute to morphine in parenteral and oral administration. *Oxymorphone* is also an effective opioid, which has higher analgesic potency than morphine when given by the oral route. It can also be used in suppository form. *Tramadol* is a codeine analog usually employed for postoperative pain. It has complex cellular effects because it acts not only as a weak μ-opioid receptor agonist but also as a norepinephrine and serotonin reuptake inhibitor. Tramadol effects last approximately 6 h.

Among phenylpiperidine derivatives, *meperidine* is a strong analgesic with less constipation effects than morphine but similar dependence liability. Its metabolite normeperidine is an excitatory agent, the accumulation of which can produce delirium, hyperreflexia, and seizures, especially in people with kidney dysfunction or who misuse meperidine at high doses.

Fentanyl and its congeners, *sufentanil, alfentanil*, and *remifentanil*, are short-acting μ-opioid receptor agonists. These drugs are highly lipid soluble and cross the blood–brain barrier easily. Fentanyl and sufentanil are used parenterally during and after surgery because of their rapid analgesic effect. Fentanyl is also available in skin patches for prolonged drug delivery and in lollipops for the short treatment of surgical pain in children (Inturrisi 2002; Trescot et al. 2008).

Table 3 Clinically relevant opiates

Drug	Use	Administration route/dose[a]	Pharmacokinetic aspects	Comments	μ	κ	δ
Morphine	Analgesic Antitussive	*p.o.*: 15–30 mg; *i.m., s.c.*: 5 mg	$t_{1/2}$: 2–3.5 h Active metabolite: M6G. First-pass metabolism	Morphine is the prototypical μ−preferring agonist	+++	+	+
Heroin	Analgesic Misused drug	*i.v., i.m.*: 5 mg *i.v.*: variable	$t_{1/2}$: 0.5 h First-pass metabolism	Morphine prodrug. More potent than morphine Deadly dose: 25 mg (*i.v.*)	+++	++	++
Hydrocodone	Analgesic Antitussive	*p.o.*: 5–7.5 mg	$t_{1/2}$: 2–4.5 h		+++	+	
Oxycodone	Analgesic	*p.o.*: 5–30 mg	$t_{1/2}$: 2.5–3 h	Frequently combined with non-opioids	+	++	
Fentanyl	Analgesic Preanesthetic medication	*p.o., s.l.*: 100–800 μg	$t_{1/2}$: 2–4 h	Available as patches and lollipops	+++		
Methadone	Analgesic Assisted maintenance Tx	*p.o.*, parenteral: 2.5 mg *p.o.*: 20–40 mg	$t_{1/2}$: 20–37 h Good absorption	NMDA receptor antagonist activity	+++		
Buprenorphine	Analgesic Assisted maintenance Tx	*i.v., i.m.*: 0.4 mg *s.l.*: 2–8 mg	$t_{1/2}$: variable *i.v.*: 3 h *s.l.*: 30 h	Partial (P) μ-receptor agonist	P	−−	
Pentazocine	Analgesic Preanesthetic medication	*i.m.*: 30–50 mg *i.v.*: 20 mg	$t_{1/2}$: 2–3 h	Agonist–antagonist	-	++	+
Butorphanol	Analgesic	*i.v.*: 0.5–2 mg *i.m.*: 1–4 mg	$t_{1/2}$: 4.5–5.6 h	Agonist–antagonist	P	+++	
Nalbuphine	Analgesic Pre- and postoperative medication	*i.v., i.m.*: 10–20 mg	$t_{1/2}$: 5 h	Agonist–antagonist	−−	++	

(*continued*)

Table 3 (continued)

Drug	Use	Administration route/dose[a]	Pharmacokinetic aspects	Comments	μ	κ	δ
Meperidine	Analgesic	*i.v., i.m.:* 50–100 mg	$t_{1/2}$: 2–4 h First-pass metabolism	It has a toxic metabolite α_{2B}-adrenoceptor agonist activity	+	+	
Naloxone	Opiate overdose	*p.o.:* 0.4–2 mg	$t_{1/2}$: 2–4 h	Receptor antagonist	- - -	- -	- -
Naltrexone	Assisted maintenance Tx Opiate overdose	*p.o.:* 50 mg	$t_{1/2}$: 4–12 h	Receptor antagonist	- - -	- -	- -

[a]Doses are variable, depending on the intensity of pain and history of opioid use

Diphenoxylate is another meperidine congener commercially available in combination with atropine for the treatment of diarrhea. *Loperamide* is used alone for the same purpose. Both compounds are effective per oral route, have low water solubility, and cannot be misused intravenously because they lack central effects.

Methadone has a much longer plasma half-life than morphine (24 h vs. 2 h) and is effective when administered by the oral route (the estimated oral to parental potency ratio is 1:2 for methadone as compared to 1:6 for morphine). Despite its slow elimination, methadone's analgesic effects last only about 4–8 h. This can lead to drug accumulation with repeated dosing. As a long-acting analgesic, methadone can be used for the treatment of chronic pain. In addition, methadone is an orally active substitute in patients with dependence to heroin.

Buprenorphine is a partial μ agonist useful in treating postoperative pain and in assisted maintenance treatment for people with opiate use disorders. Buprenorphine has less misuse liability than morphine-like drugs, but similarly to the mixed agonist–antagonists, it may precipitate withdrawal in patients who have been receiving μ-opioid receptor agonists for prolonged periods. Buprenorphine discontinuation can also result in a withdrawal syndrome that is generally less severe than that observed after stopping morphine use. Buprenorphine has a very slow rate of dissociation from its receptor, and if it produces respiratory depression, relatively large doses of naloxone are needed to reverse it. There are several commercially available presentations of buprenorphine: parenteral for the relief of moderate to severe pain, transdermal for chronic pain management, and sublingual for the treatment of opiate dependence (Inturrisi 2002; Kreek 2007).

Agonist–Antagonists

Pentazocine, butorphanol, and nalbuphine are prototypical agonist–antagonists with a lower potential than morphine-like drugs for causing respiratory depression. They produce analgesia in nontolerant patients but may precipitate withdrawal in patients who have been using morphine-like drugs.

Oral *pentazocine* belongs to the benzomorphan class of opioids; it is a κ- and δ-opioid receptor agonist as well as a weak μ-opioid receptor antagonist. Oral pentazocine is useful for the relief of mild to moderate pain and is marketed in combination with naloxone. Parenterally, pentazocine acts as a potent analgesic drug. Due to its antagonist properties, pentazocine can partially antagonize the respiratory depression and analgesic effects of morphine.

Butorphanol is a morphinan-type analgesic with high affinity for κ-opioid receptors and partial μ-agonist effects. As it occurs with κ agonists, butorphanol may produce unpleasant effects in some patients (dysphoria, nightmares, anxiety, etc.). Butorphanol is available for use by injection and as a nasal spray. There is more risk of misuse with the spray formulation.

Nalbuphine is primarily a κ agonist but also a partial μ antagonist. Due to this mixed profile, nalbuphine is a potent analgesic in opioid-naïve subjects, but may produce withdrawal symptoms when administered to patients who have been taking other opioids.

Antagonists

Naloxone has high affinity for the same receptor sites where the endogenous opioid peptides and morphine bind, but has no discernable effect in opioid-free subjects. Naloxone competes with agonists and serves as an effective antidote to overcome the respiratory suppression caused by heroin and other morphine-like analgesics. When administered to an opiate-dependent person, naloxone can also precipitate a withdrawal syndrome. Naloxone is effective only parenterally, has a short half-life, and is rapidly redistributed after injection. These characteristics limit its clinical application.

Naltrexone has a similar pharmacological profile than naloxone, but with a much more prolonged action. Because it is a competitive antagonist, naltrexone attenuates or blocks the subjective effects of intravenously administered opiates. It is used to block the rewarding effects of heroin in detoxified opiate users in case they relapse. It has also proved to be effective for the treatment of alcohol use (Inturrisi 2002; Alexander et al. 2013).

Absorption, Distribution, Metabolism, and Excretion

Morphine is slowly absorbed from the digestive system because it is a base, and as such, most of its molecules are ionized in acidic pHs. Also, morphine undergoes significant first-pass metabolism by enzymes in the digestive system, and only a small fraction can get to the brain when administered by the oral route. Opiate analgesics are frequently given orally because their low absorption can be an advantage to maintain stable drug levels in the blood. Other drugs, including nalorphine and naloxone, are poorly absorbed from the digestive system and are thus administered parenterally. Buprenorphine also undergoes substantial hepatic first-pass metabolism, and therefore sublingual, rather than oral, administration is used. The more lipophilic compounds are more rapidly absorbed. An example is methadone because despite important between-subjects variability, up to 90 % is absorbed after oral administration. Other agents with greater lipid solubility such as fentanyl can be absorbed through the skin.

When opiates are consumed for their subjective effects, parenteral routes are preferred. Heroin, for example, can be smoked, taken intranasally, or used intravenously. All three-administration routes result in rapid absorption and distribution. Because heroin is a highly lipid-soluble molecule, it enters the brain quickly and in high concentrations. After absorption, most opiates are concentrated in the lungs, liver, and spleen, and a large percentage is bound to blood proteins. Morphine is eliminated rapidly, and very low concentrations remain in the body 24 h after administration. Opiates cross the placental barrier and can induce a dependent state in the infant born to an opiate-dependent mother (Jones et al. 2010).

Heroin is a prodrug (i.e., a drug that is administered in a biologically inactive form and is biotransformed into an active metabolite) that needs to be converted first into 6-monoacetylmorphine (6-MAM) and then into morphine to produce its effects. A certain amount of codeine is also transformed into morphine by a reaction

catalyzed by the hepatic enzyme CYP2D6. Important variations in the efficiency of this enzyme exist, which can account for differences in the effects of codeine among different populations. Hydrocodone and tramadol are also prodrugs that are converted to active forms by CYP450 isoenzymes.

Morphine is metabolized by conjugation with glucuronic acid in the liver. The resulting metabolites are morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). M3G has little affinity for opioid receptors, but M6G is active and more potent than morphine itself. About 10 % of morphine is excreted in the urine unchanged. Its metabolites are eliminated in the urine and, a small proportion, in the feces.

There are important changes in the duration of action between opiates. Short half-life compounds (2–4 h) include morphine, codeine, oxycodone, hydromorphone, meperidine, fentanyl, and naloxone, among others. Compared to other analgesics, methadone has an extremely long half-life of approximately 24 h. This is due to the fact that methadone binds extensively to blood proteins and is not available for metabolism (Inturrisi 2002; Trescot et al. 2008). Other drugs with long half-life values are levorphanol and propoxyphene (Table 3).

Knowledge of pharmacokinetic properties of opiates can be used to produce useful drug combinations. A good example is a sublingual combination of buprenorphine and naloxone for opioid maintenance therapy. When the pill is administered sublingually, buprenorphine is absorbed, but naloxone is not. However, the presence of naloxone serves to discourage the illicit use of this combination as it precipitates drug withdrawal if the pill is dissolved and injected.

Opiate rotation is a common practice in pain relief. It involves substituting the opiate analgesic drug a patient is receiving with another one in order to improve pain management and/or to limiting adverse effects. Interindividual differences in sensitivity to specific compounds may be due to variations in drug-metabolizing enzyme activities, relative selectivity for opioid receptors, the presence of non-synonymous single-nucleotide polymorphisms of the μ-opioid receptor, and previous history of opiate use, among other factors. The following sections present an overview of opioid receptor subtypes and cellular effects produced by different opioid receptor ligands (Inturrisi 2002).

Opioid Receptors

Structure

Opioid peptide (OP) receptors are members of the class A (rhodopsin) family of seven transmembrane GPCRs. As all members of the family, opioid receptors have an extracellular amino terminus with glycosylation sites, seven membrane-spanning segments linked with three loops on either side of the membrane, and an intracellular carboxyl-terminal domain (C terminus) (Alexander et al. 2013). Opioid receptors also have a highly conserved pair of Cys residues in the first and second extracellular domains, which form a receptor-stabilizing disulfide bond. The intracellular regions,

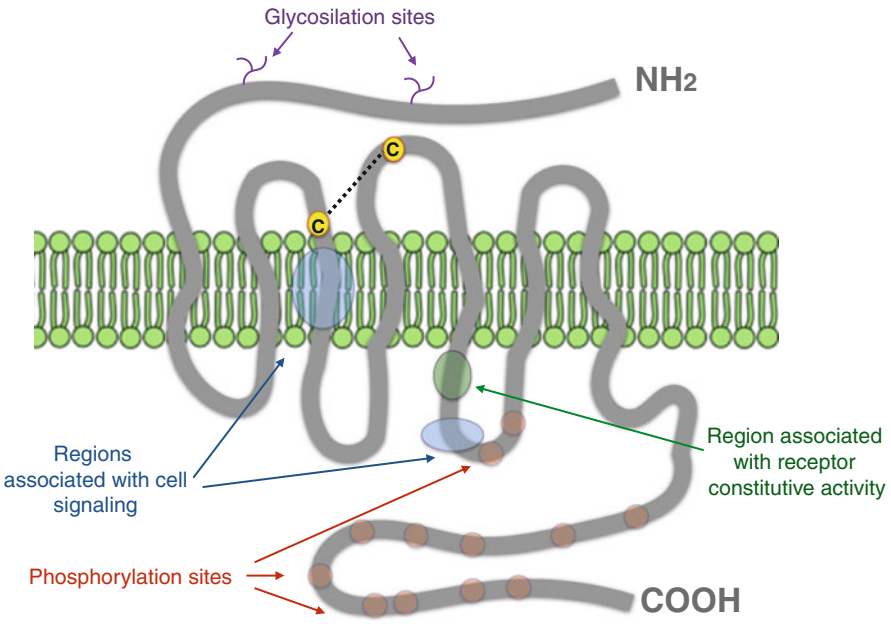

Fig. 3 Schematic representation of the μ-opioid receptor depicting some relevant structural and functional characteristics. *Yellow* circles represent the two conserved cysteine residues characteristic of the class A family of G-protein-coupled receptors

particularly the C terminus, have important phosphorylation sites involved in the receptor desensitization, internalization, and resensitization processes (Fig. 3).

Receptor Types and Subtypes

There are three opioid receptor types with close structural homologies: μ (or MOP), δ (or DOP), and κ (or KOP). The fourth member of the family is the NOP (for nociceptin opioid peptide receptor), which is considered an opioid-related receptor due to its distinct pharmacology (Alexander et al. 2013; Cox et al. 2015). Four genes designated as *Oprm1* (or MOR1), *Oprd1* (or DOR1), *Oprk1* (or KOR1), and *Oprl1* (or ORL1) codify for each receptor type. Each gene has a single regulatory pathway and a specific expression pattern.

Opioid receptors are widely distributed throughout the peripheral nervous system and CNS, mediating the diverse effects of opioid receptor agonists. All four receptor types are expressed in the spinal cord on overlapping populations of neurons. In the brain, μ, κ, and NOP receptors are found throughout the cortex, midbrain, and hindbrain; in contrast, δ-opioid receptors have a more focal distribution throughout the limbic and prelimbic brain regions (Inturrisi 2002). Specific ligands and physiological functions have been identified for each opioid receptor type, some of which are summarized in Table 4.

Table 4 Opioid receptors, distribution, ligands, and functions

Receptor	Distribution	Agonists	Antagonists	Physiological functions
μ **MOR** **MOP** **(OP_3)**	Thalamus Caudate putamen Neocortex Nucleus accumbens Amygdala Dorsal horn of the spinal cord Periaqueductal gray Brain stem	Morphine Fentanyl Sufentanil DAMGO Buprenorphine Nalbuphine Codeine Levorphanol Methadone Meperidine Endogenous: Endomorphin-1 Endomorphin-2 β-Endorphin Enkephalins	Naloxone Naltrexone Nalmefene β-Funaltrexamine Nalorphine CTAP CTOP	Analgesia, mood, respiratory and cardiovascular functions, gastrointestinal (GI) motility, feeding, locomotor activity, thermoregulation, hormone secretion, immune functions
δ **DOR** **DOP** **(OP_1)**	Similar distribution than μ with high density in olfactory areas Also present in thalamus and hypothalamus	D-Ala-deltorphin I and II DPDPE SNC80 Endogenous: Enkephalins β-Endorphin	Naltrindole Naltriben Naltrexone Naloxone	Analgesia, gastrointestinal motility, mood and behavior, olfaction, cardiovascular regulation
κ **KOR** **KOP** **(OP_2)**	Cerebral cortex Amygdala Hypothalamus Pituitary	Ketocyclazocine Bremazocine U-50488 U-69593 Butorphanol Salvinorin A Endogenous: Dynorphin A Dynorphin B	Nor-binaltorphimine GNTI Nalmefene Naloxone Naltrexone Buprenorphine	Regulation of nociception, diuresis, feeding, neuroendocrine and immune system functions
Nociceptin **NOR** **NOP** **(OP_4)**	Cortex, olfactory nucleus Ventral forebrain Hippocampus Hypothalamus Amygdala Ventral tegmental area Rostral ventromedial medulla Locus coeruleus Dorsal horn of the spinal cord	Ro64-6198 N/OFQ-(1–13) Endogenous: Nociceptin/ orphanin FQ	SB612111 J-113397 UFP-101	Regulation of nociception, autonomic control of physiological processes

Pharmacological evidence has suggested the existence of several other putative receptor subtypes, but no gene has been cloned for any of them. Recent evidence suggests that this receptor heterogeneity may result from mechanisms that include alternative splicing, single-nucleotide polymorphisms of opioid receptor genes, and receptor dimerization.

Alternative mRNA Splicing

As is the case with most genes in eukaryotic cells, genes coding for opioid receptors comprise introns and exons. After transcription, introns are removed from the pre-mRNA by a process called splicing. Sometimes an exon can be either included within or excluded from the final transcripts. Alternative splicing occurs when the mRNAs produced from a single gene have different exon composition that will eventually produce different proteins. Cloning studies have identified several splice mRNA variants of the μ-opioid receptor, which basically differ at the tip of the intracellular C terminus. The biological relevance of these splice variants is unclear; however, differences in the C-terminal tails would be expected to affect the interaction of the receptor with components of signaling, regulatory, and recycling pathways. Among the several splice mRNA variants described for the *Oprm1 or* MOR1 gene, only three (MOR1, MOR1A, and MOR1B) are widely distributed throughout the brain. There is only one study reporting the presence of a splice variant of the δ-opioid receptor in mouse brain; however, three splice mRNA variants have been described for the κ-opioid receptor.

Polymorphisms

Non-synonymous single-nucleotide polymorphisms (SNPs) produce amino acid changes, which have the potential to affect ligands' binding to their receptor site and alter the conformation of the ligand–receptor complex and/or the complex's efficacy to G-protein coupling and signaling. Particularly, SNPs in the μ-opioid receptor (*Oprm1*) gene can alter the analgesic responses to opiates in humans (Knapman and Connor 2015; Nielsen et al. 2015). Currently, more than 100 polymorphisms in the *Oprm1* gene have been identified. The effects of all these polymorphisms on opioid-induced analgesia have not been assessed. However, one of the best-studied SNPs is the A118G, which removes one glycosylation site in the N-terminal extracellular domain. This SNP reduces signaling efficacy and expression of the μ-opioid receptor and has been associated with differences in morphine use by patients. Other SNPs affecting the third transmembrane segment of the μ-opioid receptor can alter cell signaling. Potentially relevant SNPs are those coding for the R260H- and R265H-mutant receptors, because these variants show reduced basal activity of the receptors when expressed in several experimental preparations (Knapman and Connor 2015; Nielsen et al. 2015). Although it is not sufficiently studied yet, it is likely that several SNPs in opioid receptors can modify surface receptor expression, receptor trafficking and signaling, as well as alterations to ligand effects, giving rise to pharmacologically different receptor subtypes. Interestingly, SNPs are also present in the genes that codify for the opioid precursors as well as in drug transporters involved in the absorption and metabolism of opioids.

Dimerization

Many GCPRs exist as dimers. Heteromeric dimers refer to macromolecular complexes composed of at least two functional receptor units that show specific biochemical properties different from those of the individual components. Numerous studies in heterologous systems have reported that the opioid receptors form homomeric dimers, as well as heteromeric dimers (μ/δ, μ/κ, μ/NOP, δ/κ, δ/NOP, κ/NOP). Heteromeric dimers may be formed with non-opioid receptors, such as those to cannabinoids, chemokines, or glutamate (e.g., δ/CB_1, δ/CXCR4, δ/CXCR2, μ/NMDA) (Massotte 2015). In this case, activation of a single receptor is sufficient to initiate G-protein signaling.

Several receptor pairs have been co-localized in the same neurons in vivo, particularly in the dorsal root ganglia (DRG) of the spinal cord. This is the case of μ- and δ-opioid receptors, as well as adrenergic and opioid receptors (μ/α_{2C}, μ/α_{2A}, δ/α_{2A}, δ/α_{2C}). A finding worth mentioning is that μ-opioid receptor expression is reduced in DRG in knockout mice for the δ-opioid receptor. Moreover, methadone and the synthetic peptide DAMGO (both μ-opioid receptor agonists) internalize not only μ-opioid receptors, but also μ/δ dimers. In addition, some antagonists of the κ-opioid receptor act as allosteric modulators of the responses to δ agonists in δ/κ heteromeric dimers (Massotte 2015).

Some examples of opioid receptor dimerization which could account for different pharmacological profiles observed with specific agonists in various tissues are exemplified by the fact that the δ/δ or μ/μ receptor dimers are coupled to and signal via $G_{\alpha i/o}$ proteins; however, δ/μ heteromers can also be associated with G_z and/or β-arrestin 2. Also, DAMGO stimulates Ca^{2+}-mediated signaling instead of $G_{\alpha i/o}$-mediated signaling in cells expressing the δ/μ heteromers.

Cellular Signaling

G-Protein-Dependent Signaling

Acute activation of the different opioid receptor types produces similar intracellular effects. Opioid receptors are predominantly coupled to the pertussis toxin-sensitive guanosine triphosphate (GTP)-binding proteins G_i/G_o. After activation, the G protein's α_i subunit inhibits the adenylyl cyclase (AC) enzyme, reducing cAMP production and the activity of protein kinase A (PKA), which in turn affects other cell processes, including gene expression. On the other hand, the G protein's βγ subunit (which works as a single entity) increases K^+ conductance in G-protein-gated inward rectifying potassium channels (GIRKs) causing hyperpolarization. It also binds to the C terminus of Ca^{2+} channels (N-type and P/Q-type) decreasing Ca^{2+} entrance (Fig. 4). A reduction in the amount of Ca^{2+} that enters the cell during an action potential proportionately reduces the amount of neurotransmitters released (Waldhoer et al. 2004). This effect has been observed in several in vitro preparations as well as in neurons of the locus coeruleus, ventral tegmental area, hippocampus, and DRG, suggesting that ion channels are one of the most important targets for opioid receptor activation.

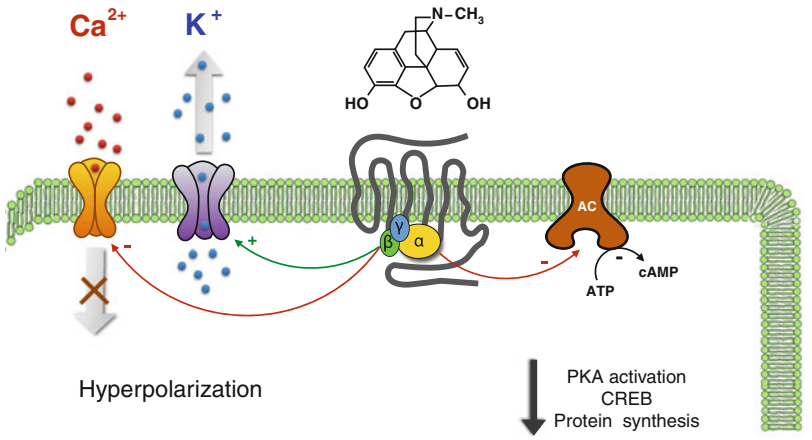

Fig. 4 Opioid receptors are predominantly coupled to GTP-binding proteins G_i/G_o. When activated, the G protein's α subunit inhibits adenylyl cyclase (*AC*), decreasing cyclic adenosine monophosphate (*cAMP*) levels and the activity of protein kinase A (*PKA*). This results in reduced phosphorylation of the transcription factor CREB (cAMP response element binding protein), thereby altering gene expression. The G protein's βγ subunit increases K^+ conductance and decreases Ca^{2+} entrance causing hyperpolarization. βγ subunit also mediates the stimulation of mitogen-activated kinase (*MAPK*) cascades

Several reports have provided evidence that G protein's βγ subunit also mediates the stimulation of mitogen-activated kinase (MAPK) cascades by opioid receptors. The MAPK pathways comprise the extracellular signal-regulated kinases (ERKs), Jun N-terminal kinases (JNKs), and p38 kinases. Among them, the effect of opioids on ERKs is the best understood. It is worth mentioning that MAPK activation occurs not only through this G-protein-dependent mechanism, but also in a β-arrestin-dependent manner (see below). The time course of the G-protein-dependent mechanism is rapid, while that depending on β-arrestin is slower.

Under certain circumstances, opioid agonists can couple to Gs. This occurs at very low doses and is usually masked by the more pronounced inhibitory actions of opioids. Very low doses of antagonists are enough to block opioid excitatory effects (Waldhoer et al. 2004).

Phosphorylation, Desensitization, and Opioid Receptor Internalization

Opioid receptors are subject to a variety of regulatory processes that include phosphorylation, desensitization, internalization, and downregulation (Williams et al. 2013). Mu-opioid receptors have more than 15 serine, threonine, and tyrosine residues, which are accessible to kinases (enzymes that add phosphate groups to target molecules and change their biological activity). Phosphorylation is carried out by various kinases, including serine/threonine protein kinases, G-protein-coupled receptor kinases (GRKs), and/or kinases activated by second messengers (such as PKC and Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII)). The specific

sites where phosphorylation takes place vary depending on the particular kinases involved. Some examples are shown in Table 5.

Recent studies suggest that phosphorylation in two amino acid clusters (between residues 375 and 379) in the μ-opioid receptor are pivotal for acute desensitization. Interestingly, different opioid receptor agonists may induce diverse degrees of phosphorylation. It seems that this may depend on individual receptors achieving a critical number of phosphorylated residues in a specific region of the carboxyl-terminal domain.

Several research groups have shown that when endogenous opioids bind to μ-opioid receptors, they are rapidly internalized by endocytosis into clathrin-coated pits. There, the peptide and receptor dissociate, and the receptor promptly returns to the cell surface to interact with other ligands. Both μ and δ receptors can be internalized in response to exogenous agonists. However, there are differences between μ and δ agonists, as well as among μ-opioid receptor ligands in their ability to induce receptor internalization; for example, methadone and fentanyl induce endocytosis, but morphine does not. In general, phosphorylation of μ, δ, κ, and probably NOP receptors promotes binding of regulatory proteins called β-arrestins, which prevent the receptors from further coupling to G proteins. These processes have different time courses. Phosphorylation takes place in about 1–2 min, whereas β-arrestin 2 recruitment and rapid desensitization take about 3–5 min. The receptors bound to β-arrestins are concentrated in clathrin-coated pits, which then undergo endocytosis into the early endosomes. Here, the receptor may follow two pathways, resensitization or endocytosis (Fig. 5). Mu-opioid receptors continue to be trafficked in endosomes, they are dephosphorylated, the ligand comes off the receptors, and then the receptors are represented in the cell membrane. In contrast, δ receptors are trafficked to lysosomes where they usually are degraded. In general, receptor binding of a ligand, without endocytosis and resensitization, contributes to downregulation of that receptor. It is important to underline that both the desensitization and the internalization processes are determined in part by the specific ligand bound to opioid receptors, as high-intrinsic activity μ agonists are more efficient to induce receptor phosphorylation than low-intrinsic activity agonists. Interestingly, internalization also depends on the kinases involved in the process of phosphorylation (Williams et al. 2013).

Table 5 Kinases and phosphorylation sites of opioid receptors to produce acute desensitization

Receptor	Kinase	Phosphorylation site	β-arrestin recruited
μ	GRK2, CaMKII, Tyrosine kinase, PKCα, PKCε, and PKCζ	Ser363, Ser370, Ser375, Tyr106, Tyr166, Ser383, Ser394	β-Arrestin 2
δ	GRK2	Ser358, Ser363	β-Arrestin1/2
κ	GRK3/GRK5	Ser369	β-Arrestin 2
NOP	GRK3	Ser363	β-Arrestin 3

GRK G-protein-coupled receptor kinase, *CaMKII* Ca^{2+}/calmodulin-dependent protein kinase, *PKC* protein kinase C

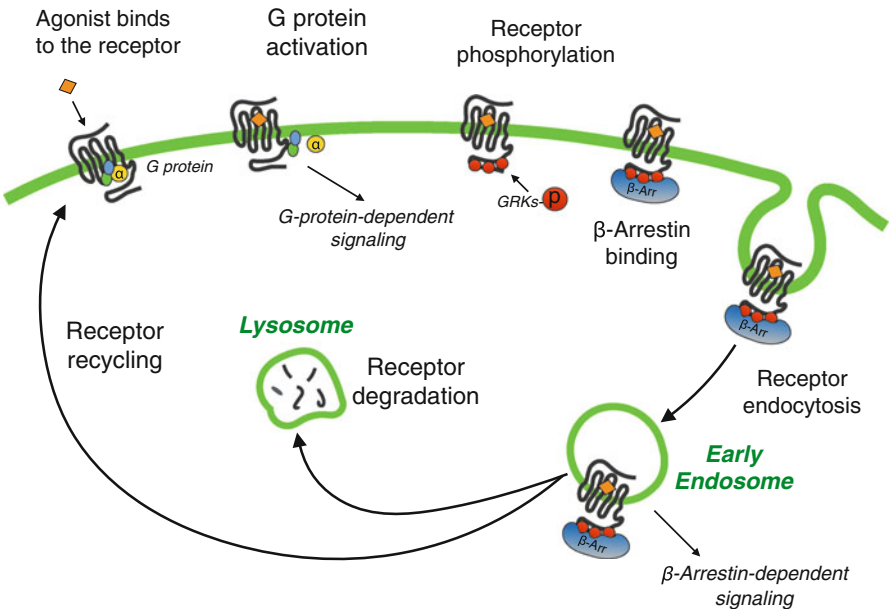

Fig. 5 Signaling through μ-opioid receptor differs between agonists. Agonist binding activates G-protein-dependent signaling. G-protein-coupled receptor kinases (*GRKs*) phosphorylate the receptor. The phosphorylated ligand–receptor complex binds to β-arrestins, which prevent further coupling to G proteins. The receptors bound to β-arrestins undergo endocytosis into the early endosomes. Once internalized, the receptor can be resensitized and replaced in the cell membrane for further activation or trafficked to lysosomes for degradation. Several agonist–receptor complexes that promote internalization favor β-arrestin-dependent activation of MAPK cascades

β-Arrestin-Dependent Signaling

As previously mentioned, opioid receptor-signaling pathways involve not only $G_{i/o}$ protein activation, inhibition of cAMP formation, and subsequent regulation of PKA activity, but also activation of MAPKs and other enzymatic effectors. These signals are transmitted from the ligand–receptor complex to the nucleus through the cytoplasm by several protein–protein interactions. Studies over the past years have shown that β-arrestins, acting as scaffolding proteins, can switch the coupling of opioid receptors from the acute regulation of ion channels' conductance to a different mode of signaling involving MAPK cascade activation associated to long-term changes in cellular function, such as cell proliferation, differentiation, and synaptic plasticity.

Various opioid-receptor agonists activate kinase cascades, which include members of the MAPK family and phospholipase D (PLD) to produce receptor desensitization. High-intrinsic activity μ agonists (DAMGO, β-endorphin, methadone, or fentanyl), but not low-intrinsic activity μ agonists (morphine, buprenorphine, or oxycodone), stimulate phospholipase PLD2. Because PLD activation mediates μ-opioid receptor internalization, endocytosis is a function not only of the receptor but also of the agonist bound to it.

Activation of MAPKs is also dependent on the agonist–receptor complex; for example, fentanyl, but not morphine, activates ERK1/ERK2 in a β-arrestin-

dependent manner. The mechanisms of ERK1/ERK2 to produce desensitization are unknown; however, it has been proposed that these kinases phosphorylate μ-opioid receptors at sites not occupied by $G\alpha_i$ subunits, thus preventing receptor–effector coupling. Recently, it has been demonstrated that p38 MAPK activation facilitates μ-opioid receptor internalization by enhancing the function of endocytic machinery (Raheal et al. 2011). Activation of MAPKs by κ- and δ-opioid receptors has also been documented. There is evidence that the ERK pathway plays an important role in the cellular and molecular mechanisms underlying drug dependence.

Additional Regulatory Mechanisms of Opioid Receptors

Biased Agonism

Biased agonism refers to the ability of different agonists to differentially activate signaling cascades or regulatory events, including differences in receptor trafficking. This implies the formation of different protein complexes activated by the ligand binding to the receptor, which in turn triggers different effects. Biased agonism has led to the hypothesis that specific drugs catch the receptors with the precise conformation that elicits specific downstream events. Different agonists have bias for G-protein interaction versus the β-arrestin 2 recruitment pathway. For instance, some morphine metabolites, including M6G, show lower potencies for G-protein activation, but higher potencies and efficacies for β-arrestin 2 recruitment than morphine. DAMGO recruits β-arrestin 1 and β-arrestin 2, while morphine only recruits β-arrestin 2. Furthermore, it has been described that β-arrestin 2 knockout mice exhibit strong analgesia with reduced respiratory and gastrointestinal side effects (Kelly 2013). This highlights the importance of identifying biased agonists to select only some desirable effects mediated by the μ-opioid receptor

Allosteric Regulation

As mentioned above, certain ligands can bind to sites on opioid receptors that are separate (allosteric) from the orthosteric site, while orthosteric ligands bind to the same site on the receptor that recognizes an endogenous agonist. Opioid receptors, like other GPCRs, exist in at least two conformations: constitutively inactive (R) and constitutively active (R*). Orthosteric and allosteric ligands binding to their respective binding sites in the opioid receptor can stabilize one receptor state at the expense of the other.

Some molecules may behave as positive or negative allosteric modulators of opioid receptors. For example, positive allosteric modulators (or PAMs) of the μ-opioid receptor have little agonist activity on their own, but enhance the affinity, potency, and maximal response of endogenous μ-opioid receptor agonists (Burford et al. 2015). Negative allosteric modulators (NAMs) have no intrinsic agonist efficacy; however, they bind to the receptor and inhibit the binding affinity and/or efficacy of orthosteric agonists.

It has been known, since 1973, that sodium is a NAM because of its capacity to decrease the binding of agonists to μ-opioid receptors. Interestingly, it is now recognized that sodium inhibits about 65 % the agonist binding and signaling in

μ-and δ-opioid receptors, but only 20 % in κ-opioid receptors. Furthermore, other cations such as potassium and lithium also reduce agonist binding to the δ-opioid receptors. Contrariwise, manganese restores full agonist binding in the presence of sodium. It is believed that sodium affects the equilibrium between R and R* by binding to an aspartate residue in transmembrane helix 2, modulating the binding of endogenous orthosteric ligands.

The research of the effects of PAMs for the opioid receptors is just starting. However, it is likely that PAMs may avoid receptor downregulation and other compensatory mechanisms, which are triggered by sustained opioid-receptor activation produced by orthosteric agonists. Based on this, it has been speculated that PAMs could preserve the activity of the endogenous opioid peptides and produce less tolerance and dependence than exogenous orthosteric agonists. It is also likely that low doses of PAMs combined with clinical opiates could provide therapeutic benefit with fewer side effects.

Epigenetic Regulation

All opioid receptors are subjected to epigenetic regulation because their coding genes are rich in CpG islands, which can be highly methylated. DNA methylation in cytosine nucleotides suppresses gene transcription. Several transcription factors regulate the activity of the opioid receptor gene *Oprm1*, among which the best studied is CREB (cAMP response element binding protein), but there are others. Also, the *Oprm1* gene is repressed by various transcription factors (Oct-1 or octamer-1, among others). Therefore, DNA methylation can profoundly modify μ-opioid receptor expression. Moreover, the *Oprm1* gene can also be modulated by microRNA (miRNA). Epigenetic regulation of coding genes for δ-, κ-, and NOP receptors has also been described. Interestingly, the opioid peptide precursors (proenkephalin, POMC, prodynorphin, and pronociceptin genes) are also epigenetically regulated by DNA methylation and histone methylation (Muñoa et al. 2015).

Epigenetic regulation along with heteromerization, allosteric modulation, and biased agonism combined with various expression patterns and differing selectivity for opioid receptor subtypes may produce an enormous diversity in the physiologic processes related to activation of opioid receptors (Fig. 6).

Addiction, Physical Dependence, and Tolerance

So far, we have discussed opioids mostly as clinical useful drugs, but they are equally important as misused and addictive psychoactive substances. Some epidemiological data illustrate this point. According to the more recent World Drug Report, there are approximately 32.4 million opiate users worldwide. In North America, the prevalence is 3.8 %. Additionally, opium poppy cultivation reached historically high levels in 2014 since the late 1930s, and the number of opiate-related deaths has reached the highest level in a decade and continues to rise in the United States. Addiction (drug dependence), physical dependence, tolerance development, and overdose deaths are the main adverse effects associated with opiate use.

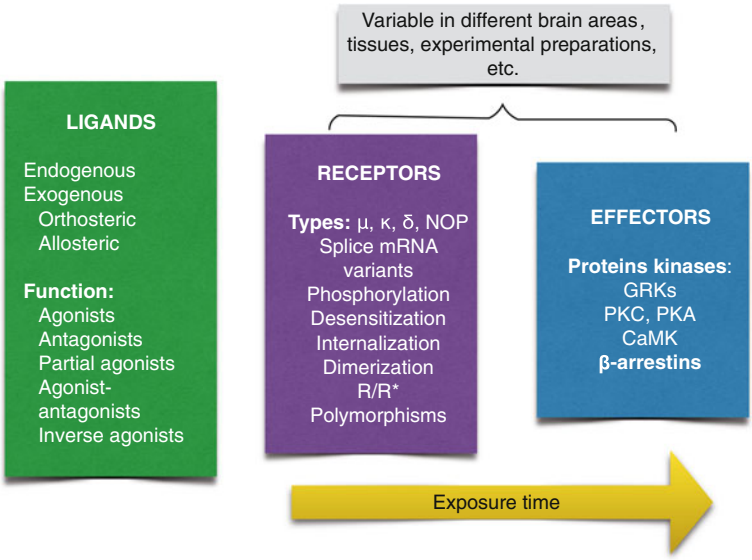

Fig. 6 The outcome of opioid receptor activation depends on the ligand, receptor type, receptor state, and regulatory proteins available in a given tissue or experimental preparation. Opioid exposure time and history of previous opioid also determine the resulting effect

Opioid misuse (abuse) can occur with prescription medications and illegal drugs. Behaviors involved in misuse of prescription opioids include injecting oral formulations, obtaining drugs from nonmedical sources, forging prescriptions, frequent prescription refills, and escalating doses without medical authorization. A relatively few number of opioid users develop an *addiction*. This condition is characterized by a maladaptive pattern of drug use with impaired control over use, compulsive self-administration, continued use despite negative consequences, and drug craving.

Although related, addiction is not synonymous with tolerance or physical dependence (Kreek 2007). *Tolerance* is a state in which there is a decreased response to doses that were once effective. As a result, doses are escalated in an attempt to achieve the same effects that the user experimented initially. In several instances, full recovery is not possible, and increasing the dose only enhances the probability of experiencing adverse effects. Tolerance does not develop equally to all of opioid actions and depends on the amount of drug used and the interval between doses; the more frequent the administrations, the easier for tolerance to develop. The first effects to decrease are euphoria and analgesia, followed by nausea and vomiting. Tolerance to low gastrointestinal motility and pupil constriction, on the other hand, is slow and incomplete. As a consequence, constipation may be a persistent problem in patients undergoing chronic pain treatment, and pinpoint pupils can be observed even in very chronic opiate users.

Compulsive use is often, but not always, accompanied by *physical dependence*. When a subject is physically dependent to opiates, sudden discontinuation results in

a very uncomfortable *withdrawal syndrome*. Some of the early symptoms are similar to flu and include myalgia, joint pain, lacrimation, rhinorrhea, sneezing, fatigue, dysphoria, fever, and piloerection (gooseflesh). Other symptoms are sweating, nausea, vomiting, continuous yawning, diarrhea, stomach cramps, and increased blood pressure, heart rate, and temperature. Involuntary movements, particularly of the feet, can occur, which is the reason why withdrawal is also called "kicking the habit." Once the initial stage of withdrawal is past (usually within a week), several symptoms that include craving for the drug and altered response to stress may persist for weeks and months after the last dose (Kreek 2007).

Withdrawal symptoms tend to be opposite to those produced by the agent that induced the dependent state. For instance, the analgesia and miosis characteristic of opioids are replaced by hyperalgesia and mydriasis during abstinence. Although very uncomfortable, withdrawal symptoms are usually not life threatening, except to the fetus of a mother dependent on opiates. This is why pregnant heroin users should receive assisted maintenance programs with buprenorphine or methadone. The signs of neonatal abstinence syndrome include irritability, tremors, high-pitched crying, and feeding problems. There are also hyperactive reflexes, diarrhea, excessive sucking, increased muscle tone, sneezing, yawning, vomiting, fever, restlessness, short periods of sleep, and slow body weight gain. Treatment of newborns depends on the drug used during pregnancy and the infant's overall health and may include dehydration management, special care and feeding programs, or medications (Jones et al. 2010).

Pharmacokinetic variables are important in the magnitude and duration of the withdrawal syndrome. If a competitive antagonist such as naloxone is administered, it rapidly displaces the opiate agonist from its receptor, and a more intense and shorter-lasting abstinence response is precipitated than when the opiate is discontinued. Also, the signs and symptoms may be different if the drug is short acting, like heroin, or long acting, like methadone. After abrupt cessation of heroin, there is a rapid onset of withdrawal symptoms (within 6–12 h after the last dose) that reach maximum intensity in the next two days and decline afterwards. The long-acting drug methadone and buprenorphine (a partial agonist, which slowly dissociates from μ-opioid receptors) can be used to manage withdrawal signs following chronic heroin use or in patients who have received opiates for chronic pain treatment.

It is possible to be physically dependent on a drug without being addicted to it. For example, patients who take opioids for pain control can become tolerant and physically dependent on the drug but that does not mean that they would compulsively seek out the drug when it is no longer needed for analgesia. In such cases, the clinical approach could be to gradually taper the dose instead of abrupt discontinuation to diminish the discomfort associated with withdrawal. The opposite is also true, a patient who has been recently detoxified from heroin may no longer be experiencing withdrawal symptoms but will continue to crave for the subjective effects of the opiate and may relapse to active misuse despite being aware of its negative consequences.

Although physical dependence, tolerance, and addiction are difficult to separate, there is evidence that different CNS regions are involved in these processes; for example, the mesolimbic system plays a crucial role in the case of self-administration, and the locus coeruleus and periaqueductal gray, in physical dependence and withdrawal.

The role of the mesolimbic system in opiate addiction has been extensively studied. As mentioned, opiates hyperpolarize cells via activation of $G_{i/o}$ proteins reducing neurotransmitter release. However, opiates like all addictive drugs increase the levels of dopamine in the mesolimbic system, which is a key detector of rewarding stimuli. This is an indirect effect, through inhibition of GABAergic neurons that exert a tonic inhibition of dopaminergic neurons in the ventral tegmental area (VTA). The inhibition of an inhibitory effect results in an increase in the amount of dopamine released from the VTA to the striatum and the frontal cortex.

The role of the locus coeruleus (the major noradrenergic nucleus in the brain) in physical dependence is also well described. Acutely, opiate agonists inhibit AC activity decreasing the conversion of ATP into cAMP, but this effect diminishes with time due to compensatory cellular adaptive changes, which include increased expression of certain types of AC, PKA, and the transcription factor CREB. Morphine withdrawal and opioid receptor blockade with antagonists produce an even higher increase in cAMP levels, a phenomenon described as cAMP overshoot or superactivation of adenylyl cyclase (Zhang et al. 2013). Increased cAMP activates PKA and CREB-dependent gene transcription. This effect has been observed in the locus coeruleus, periaqueductal gray, and several cell culture systems. The AC pathway hyperactivation is associated with many of the withdrawal signs such as nausea, vomiting, cramps, sweating, and increased blood pressure and heart rate. In fact, administration of the α_2 adrenergic agonist clonidine, which inhibits AC activity, can alleviate most of these effects (Kreek 2007).

Generally speaking, tolerance and dependence develop because the inhibitory μ-opioid receptor mechanisms of action become less important, and their excitatory effects become more pronounced. It is well known that potassium channels and calcium channels play an important role in the neurodepressant effects of opioid agonists. After morphine chronic treatment, potassium channel gating changes markedly, decreasing its open probability. Furthermore, there is a reduction in the effectiveness of opioid agonists to inhibit calcium channels during tolerance. Particularly, neurons from chronic morphine-treated mice show a significant reduction in P/Q-type and L-type-mediated Ca^{2+} currents. Accordingly, blockade of these channels reduces morphine tolerance.

Several other mechanisms have been shown to play important roles in the development of tolerance, including opioid receptor desensitization and trafficking (Williams et al. 2013). It has been shown that high-intrinsic activity compounds (e.g., DAMGO, fentanyl, etorphine, and methadone), but not morphine, induce μ-opioid receptor desensitization and internalization. The apparent lack of morphine's efficacy to internalize the receptor may be due to its inability to induce

receptor phosphorylation by GRK2. Endocytosis seems to be an effective way to rapidly stop G-protein-dependent signaling and allow the receptors to resensitize; as when endocytosis does not take place, other longer-lasting intracellular adaptations occur to regain homeostasis. In fact, it has been proposed that measurement of relative agonist signaling versus endocytosis (RAVE) could be an index to predict tolerance liability. Accordingly, the ability of morphine to induce changes in intracellular signaling, along with its poor ability to internalize receptors, would confer this agonist a high RAVE value and thus a high potential to induce tolerance. Contrariwise, DAMGO and etorphine, which combine high-intrinsic activity with high internalization potency, would lead to a low RAVE value and a low potential for tolerance development.

Another hypothesis proposes that repeated exposure to various opioid receptor agonists induces an overexpression of R*. When this happens, subtle alterations in the structure and/or conformation of opioid receptors can occur that reveal negative intrinsic activity of ligands previously shown to possess only neutral antagonist properties. According to some authors, in naïve, untreated systems, there are very low concentrations of R*. With repeated agonist administration, more receptors would be in the active state, whereupon an agonist will have minimal or no further effect, while an inverse agonist (such as naloxone) would be very effective (changes from R* to R will be large) (Williams et al. 2013). Such receptor adaptations to prolonged agonist exposure have been reported for μ- and δ-opioid receptors and might contribute to tolerance and withdrawal.

Functional and gene expression studies suggest that several proteins, sites, and systems are involved in the in vivo adaptations to chronic opiate exposure. Particularly, chronic morphine administration produces neuroplastic changes involving the upregulation of pronociceptive systems. How these changes are started is unknown. However, there is evidence that activation of the rostral ventromedial medulla (RVM) plays an important role in this process. For instance, the sustained administration of morphine increases the proportion of "on-cells" (cells that allow nociceptive stimulus to be transmitted) and decreases the proportion of neutral cells recorded in the RVM of rats. These data, along with evidence demonstrating that cholecystokinin (CCK) activates on-cells in the RVM, point to this peptide as an important agent in the development of opiate analgesic tolerance. In support to this point, sustained morphine administration induces upregulation of CCK mRNA and CCK release in the spinal cord, and coadministration of morphine with CCK antiserum or CCK_2 receptor antagonists reduces or prevents tolerance development.

Preclinical data have shown that repeated intrathecal injection of μ-opioid receptor agonists for several days produces tactile allodynia and antinociceptive tolerance along with elevated dynorphin content in the spinal cord. Dynorphin promotes presynaptic release of excitatory neurotransmitters (calcitonin gene-related peptide (CGRP), substance P, aspartate, and glutamate) in tolerant rats. There is also sound evidence that the activation of the glutamate NMDA receptor subtype plays an important role in opiate tolerance, since competitive and noncompetitive NMDA receptor antagonists or antisense oligodeoxynucleotides retard the development of tolerance to morphine.

A growing body of evidence suggests that the δ-opioid receptor is important for the development of tolerance to morphine (Stockton and Devi 2012; Fujita et al. 2015). For example, concurrent administration of δ-opioid receptor antagonists with morphine, or antisense oligodeoxynucleotides directed against the δ-opioid receptor, partially blocks the development of tolerance to morphine (Fujita et al. 2015). This could be due to the formation of μ/δ heteromers, which switch to the β-arrestin 2-dependent signaling cascade, but more studies are needed to clarify the cellular mechanisms underlying these effects.

Chronic morphine treatment has also been associated with activation of microglial and astrocytic cells, which may lead to spinal release of prostaglandins, CGRP, and pro-inflammatory cytokines, all of which would contribute to opiate tolerance.

It is worth mentioning that physical dependence is not directly related to tolerance. For example, concomitant treatment of morphine with CCK antagonists prevents the development of tolerance to morphine-induced analgesia but does not modify the occurrence of physical dependence. Similar results have been reported using PKC or PKA inhibitors in mice. Moreover, morphine produces physical dependence with little tolerance development in β-arrestin knockout mice.

Outlook

We have now a more comprehensive understanding of the complexity of opioid effects which depend, at least, on (a) the specific ligand used; (b) the quality, quantity, and state of the receptor mediating the response; (c) the enzymatic proteins and ion channels available in a given tissue; (d) the time of opioid exposure; and (e) the frequency of opioid administration (Fig. 6).

As the field of opioid research continues to evolve, there will be more science-based elements, which will help identify agonists or opioid analgesic combinations with the desired effects of morphine, but devoid of significant adverse effects. Promising findings will derive from the identification of positive allosteric modulators and biased ligands for the μ-opioid receptor, as well as a deeper understanding in the differences of opioid effects in populations with various SNPs of the human μ-opioid receptor. Ironically, at the same time there is a growing countermovement aimed to develop more addictive compounds. Among the new psychoactive substances synthesized in underground laboratories, there are several opiates based on the fentanyl molecule. Of particular concern is the compound MPTP (*N*-**m**ethyl-4-**p**henyl-1, 2, 3, 6-**t**etrahydro**p**yridine), which can be a byproduct of attempted meperidine synthesis. MPTP containing powder can be sold as "synthetic heroin," and aside from the negative effects associated with opiate misuse, it can induce irreversible parkinsonism due to a neurotoxic effect in the substantia nigra. Another threat comes from attempts to synthetize potent psychoactive opiates from commercially available compounds as in the case of "krokodyl" (impure desomorphine synthetized from codeine). In this way, what have been relatively safe marketed presentations can become precursors for homemade contaminated drugs.

Adequate pain management, particularly in chronic patients, continues to be a significant challenge, which requires education programs for the effective use of opioids and appropriate public policies. According to a recent report from the United Nations, the vast majority of morphine is used by a low percentage of the population living in very few countries (the United States, Canada, Western Europe, Australia, and New Zealand), and there are still over 5.5 billion people with limited or no access to opiate medications for proper pain relief treatment.

On the other hand, we are facing an epidemic of drug prescription misuse where opiates are available, with an alarming increase of overdose deaths. This underlies the need to join efforts from researchers and prevention and treatment professionals to address this problem.

References

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, Peters JA, Harmar AJ, CGTP Collaborators (2013) The concise guide to pharmacology 2013/14: G protein-coupled receptors. Br J Pharmacol 170:1459–1581

Booth M (1997). Opium. A history. Simon and Schuster Ltd, New York

Burford NT, Traynor JR, Alt A (2015) Positive allosteric modulators of the μ-opioid receptor: a novel approach for future pain medications. Br J Pharmacol 172:277–286

Cox BM, Christie MJ, Devi L, Toll L, Traynor JR (2015) Challenges for opioid receptor nomenclature: IUPHAR review 9. Br J Pharmacol 172:317–323

Fujita W, Gomes I, Devi LA (2015) Heteromers of μ-δ opioid receptors: new pharmacology and novel therapeutic possibilities. Br J Pharmacol 172:375–387

Hutchinson MR, Shavit Y, Grace PM, Rice KC, Maier SF, Watkins LR (2011) Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. Pharmacol Rev 63:772–810

Inturrisi CE (2002) Clinical pharmacology of opioids for pain. Clin J Pain 18:S3–S13

Jones HE, Kaltenbach K, Heil SH, Stine SM, Coyle MG et al (2010) Neonatal abstinence syndrome after methadone or buprenorphine exposure. N Engl J Med 363:2320–2331

Kelly E (2013) Efficacy and ligand bias at the μ-opioid receptor. Br J Pharmacol 169:1430–1446

Knapman A, Connor M (2015) Cellular signalling of non-synonymous single-nucleotide polymorphisms of the human μ-opioid receptor (OPRM1). Br J Pharmacol 172:349–363

Kreek MJ (2007). Neurobiology of opiates and opioids. In: Galanter M, Kleber HD (eds), The American Psychiatric Textbook of Abuse Treatment: 4th edition. pp: 247–259.

Massotte D (2015) In vivo opioid receptor heteromerization: where do we stand? Br J Pharmacol 172:420–434

Molina-Martínez LM, González-Espinosa C, Cruz SL (2014) Dissociation of immunosuppressive and nociceptive effects of fentanyl, but not morphine, after repeated administration in mice: fentanyl-induced sensitization to LPS. Brain Behav Immun 42:60–64

Muñoa I, Urizar I, Casis L, Irazusta J, Subiran N (2015) The epigenetic regulation of the opioid system: new individualized prompt prevention and treatment strategies. J Cell Biochem 16:2419–2426

Nielsen LM, Olesen AE, Branford R, Christrup LL, Sato H, Drewes AM (2015) Association between human pain-related genotypes and variability in opioid analgesia: an updated review. Pain Pract 15:580–594

Raheal KM, Schmid CL, Groer CE, Bohn LM (2011) Functional selectivity at the μ-opioid receptor: implications for understanding opioid analgesia and tolerance. Pharmacol Rev 63:1001–1019

Sharp BM (2006) Multiple opioid receptors on immune cells modulate intracellular signaling. Brain Behav Immun 20:9–14

Snyder SH, Pasternak GW (2012) Historical review: opioid receptors. Trends Pharmacol Sci 24:198–205

Stockton SD Jr, Devi LA (2012) Functional relevance of μ-δ opioid receptor heteromerization: a role in novel signaling and implications for the treatment of addiction disorders: from a symposium on new concepts in mu-opioid pharmacology. Drug Alcohol Depend 121:167–172

Trescot AM, Datta S, Lee M (2008) Opioid pharmacology. Pain Physician 11:S133–S153

Waldhoer M, Bartlett SE, Whistler JL (2004) Opioid receptors. Ann Rev Biochem 73:953–990

Williams JT, Ingram SL, Henderson G, Chavkin C, von Zastrow M, Schulz S, Koch T, Evans CJ, Christie MDJ (2013) Regulation of μ-opioid receptors: desensitization, phosphorylation, internalization, and tolerance. Pharmacol Rev 65:223–254

Zhang L, Loh HH, Law PY (2013) A novel noncanonical signaling pathway for the μ-opioid receptor. Mol Pharmacol 84:844–853