3

Molecular Aspects of Opioid Receptors and Opioid Receptor Painkillers

Austin B. Yongye and Karina Martínez-Mayorga
Torrey Pines Institute for Molecular Studies, Port Saint Lucie, FL, USA

1. Introduction

The unpleasant sensation of pain is experienced by all human beings at a given point in life. When pain gets severe and/or chronic it requires medical treatment. For over a thousand years, opioid agonists have been employed therapeutically to treat pain, with the first reports of such use involving the alkaloid morphine dated to the second century B.C.(Waldhoer, Bartlett et al. 2004) The term *opioid* refers to any substance with opium-like activity. Opium is extracted from the juice of the poppy plant *Papaver somniferum*. Opium contains in excess of 20 different alkaloids, and for centuries its crude form was used for pain management and for its psychological effects. In 1806 the German pharmacist Sertürner isolated a pure substance from opium, which he called morphine after the Greek god of dreams, Morpheus. Thereafter other alkaloids such as codeine (1832) and papaverine (1848) were isolated.(Reisine and Pasternak 1996) These discoveries paved the way for the use of pure alkaloids as opposed to crude opium in the medical profession. It became apparent that these alkaloids had a high potential for abuse and addiction. However, it was not until 1973 that the first descriptions of the pharmacological properties of morphine, along with other agonists and antagonists, at the level of the receptor were reported.(Pert, Pasternak et al. 1973)

Opioid receptors are of therapeutic relevance because they constitute the primary targets in the clinical treatment of both acute and chronic pain. They are members of the superfamily of seven helix transmembrane (TM) proteins known as G-protein coupled receptors (GPCRs); so-called because they are coupled in the cytoplasmic side to a group of G_i/G_o hetero-trimeric proteins called G-proteins: G_α, G_β and G_γ.(Eguchi M 2004) Currently four types of opioid receptors have been identified: μ (mu for morphine), κ (kappa for ketocyclazocine), δ (delta for deferens given that it was originally discovered in the vas deferens of mice)(Waldhoer, Bartlett et al. 2004) and orphan opioid receptor-like 1. They are in turn sub-divided into additional subtypes on the basis of their ligand binding and pharmacological profiles: μ_1-μ_2, κ_1-κ_3, and δ_1-δ_2.(Pasternak 1993; Blakeney, Reid et al. 2007) The μ, κ and δ main types are the most studied, each playing a different role in pain sedation: the μ-receptor generates the most profound analgesia, but is also associated with constipation, respiratory depression, euphoria, tolerance, dependence and addition;(Schmauss and Yaksh 1984; Cowan, Zhu et al. 1988) the δ-receptor is involved in pain relief from thermal sources,(Mansour, Khachaturian et al. 1988) but like the μ-receptor, it is also associated with respiratory depression and addiction;(Abdelhamid, Sultana et al.

1991; Maldonado, Negus et al. 1992) the κ-receptor mediates pain originating from chemical stimuli,(Leighton, Johnson et al. 1987; Wollemann, Benyhe et al. 1993) but it promotes dysphoria, diuresis and sedation.(von Voigtlander, Lahti et al. 1983; Lahti, Mickelson et al. 1985) There is also evidence that opioid receptors exist as homo- or hetero-oligomeric complexes and that their pharmacological responses may be cross-modulated.(Zhu, King et al. 1999; Rutherford, Wang et al. 2008) For instance, Waldhoer M et al. used 6'-GNTI to demonstrate the existence of a δ-κ hetero-dimer *in vivo*.(Waldhoer, Fong et al. 2005) Furthermore, δ-opioid antagonists suppress some of the side effects of μ-opioid agonists such as dependence and tolerance while retaining their analgesic properties.(Ananthan 2006) The realization of this potential for cross-modulation generated interests in developing so-called bivalent ligands of opioid receptors.(Dietis, Guerrini et al. 2009; Balboni, Salvadori et al. 2011) One therapeutic relevance of opioid receptors worth mentioning is that opioid receptors antagonists such as naloxone are utilized clinically in the treatment of morphine and heroin addiction and overdose.(Blakeney, Reid et al. 2007) In this chapter, we summarize structural aspects of opioid receptors and opioid receptor ligands, with special emphasis on the μ-opioid receptor. The importance of the combined use of experimental information and computational models is highlighted.

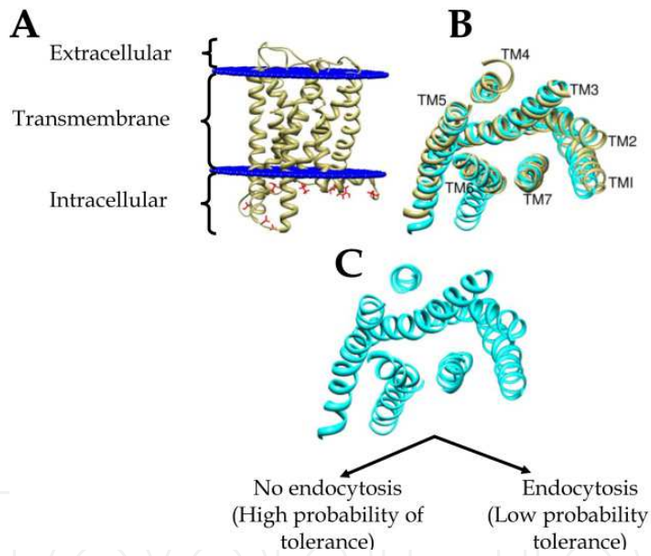


Fig. 1. (A) The three domains of the μ-opioid receptor. Intracellular serine, threonine and tyrosine residues are shown in red. (B) Extracellular perspective: the seven transmembrane helices are arranged sequentially in a counterclockwise direction. The modeled active and inactive structures are shown in cyan and tan, respectively. A substantial structural difference between the two states can be seen at TM6. (C) Hypothesized outcome of degree of ligand-induced receptor endocytosis. Homology models from Pogozheva, I. D., A. L. Lomize, et al. (1998), Fowler, C. B. et al. (2004).

2. Biochemical and biophysical characterization of the μ-opioid receptor

2.1 Structural studies of the μ-opioid receptor

The notion of preferential stabilization of distinct conformational states by agonists and non-agonists has been established experimentally and also demonstrated computationally

(see Figure1). The experimental studies include: Li *et al.* (Li, Han et al. 2007) employing agonists and inverse-agonists of the muscarinic acetylcholine GPCR; Xu *et al.* (Xu, Sanz et al. 2008) identified inter-residue interaction differences between the active and inactive states for the μ-opioid receptor. From the computational side, molecular dynamics simulations studies suggest that μ-opioid receptor agonists and antagonists bind to the receptor with a set of interactions that are specific to each class.(Kolinski and Filipek 2008) In addition, MD simulations have been utilized to elucidate an increase in solvent exposure of the intracellular domains between helices 3 and 6, and different interactions between the arginine of the E/DRY motif for active and inactive GPCRs.(Fanelli and De Benedetti 2006)

2.2 Mechanism of activation of opioid receptors

To describe the mechanism of activation and action of opioid receptors it suffices to describe the cellular assembly of these receptors. Opioid receptors comprise three domains: an extracellular N-terminus, seven transmembrane α-helices and an intracellular C-terminus, Figure 1. The 7TM helices are arranged sequentially in a counter-clockwise manner when viewed from the extracellular side, and are linked by loops called EL1, EL2, EL3, IL1, IL2 and IL3. EL and IL denote extracellular loop and intracellular loop, respectively. Across the receptors the intracellular loops share the highest sequence homology (90%), followed by TM domains (70%), while the extracellular loops, the N- and C-termini show the greatest diversity.(Knapp, Malatynska et al. 1995) Coupling between the receptors and G-proteins occurs via the pertussis toxin sensitive G_α unit.

Activation and signaling from opioid receptors by different classes of ligands are regulated by a highly conserved mechanism.(Finn and Whistler 2001; Eguchi 2004) They are activated naturally by endogenous peptides, but also by exogenous opiates. Agonist-dependent opioid receptor activation induces conformational changes in the receptor, which promote exchange of G_α-bound GDP for unbound GTP, followed by dissociation of the G-proteins from the receptor. The G_α unit further dissociates from the $G_{\beta\gamma}$ units. Signal transduction occurs via GTP-bound G_α inhibiting adenylate cyclase, responsible for producing cyclic adenosine monophosphate (cAMP). Down-regulation of cAMP results in the reduction of voltage-dependent current and neurotransmitter release.(Eguchi 2004) Moreover, the threshold of voltage-dependent ion channels becomes more negative, decreasing inward flow of current responsible for spontaneous neuronal activity resulting in a drop in cellular excitability. cAMP reduction also leads to a decrease in neurotransmitter release by cAMP-dependent protein kinase. The G_β and G_γ subunits also play key roles in decreasing cell excitability by inhibiting voltage-gated Ca^{2+} channels, hyperpolarizing the membrane and up-regulating the conduction of potassium.(Eguchi 2004) These combined decreases in neurotransmitter release and excitability are manifested as analgesia. Finally, the inactive state is re-constituted when G_α-bound GTP is hydrolyzed to GDP, re-association with $G_{\beta\gamma}$ and recoupling with the receptor.

Numerous experimental approaches have been utilized to investigate GPCR structure and activation including: solution and solid-state NMR, fluorescence, IR and UV spectroscopy, spin-labeling, site-directed mutagenesis, substituted cysteine accessibility, disulphide cross-linking, engineering metal-binding sites, and identification of constitutively active mutants.(Gether 2000; Meng and Bourne 2001; Parnot, Miserey-Lenkei et al. 2002; Decaillot, Befort et al. 2003; Hubbell, Altenbach et al. 2003; Struts, Salgado et al. 2011) Experimentally and computationally, the importance of the lipid membrane should be recognized. It is well

documented that membrane composition affects receptor function.(Botelho, Gibson et al. 2002; Botelho, Huber et al. 2006) From the computational side, molecular dynamics simulations showed that the modification of the original positioning of the lipids in the membrane influences the dynamics of the protein.(Lau, Grossfield et al. 2007) In addition, water flux though the transmembrane helices, has been proposed to affect rhodopsin activation. (Grossfield, Pitman et al. 2008) Lastly, the time scale involved in the activation of GPCRs is a challenging task. However, the combined use of computer power and experimental information allows for the generation of detailed structural information. For instance, 2000 ns molecular dynamics simulations and solid-state 2H-NMR data were combined to elucidate the protonation state of key residues directly involved in rhodopsin activation.(Martinez-Mayorga, Pitman et al. 2006) This exemplifies how computational models can provide detailed structural information not available otherwise.

Advances in crystallography and molecular engineering have provided the three-dimensional structures of a few GPCR's: rhodopsin,(Palczewski, Kumasaka et al. 2000; Ridge and Palczewski 2007; Choe, Kim et al. 2011) β-adrenergic receptor,(Kobilka and Schertler 2008) and adenosine receptor.(Jaakola, Griffith et al. 2008) In the absence of experimental structures of opioid receptors, the 2.6-Å resolution crystal structure of bovine rhodopsin(Palczewski, Kumasaka et al. 2000) has served as a template for generating homology models of these receptors,(Pogozheva, Lomize et al. 1998; Fowler, Pogozheva et al. 2004; Fowler, Pogozheva et al. 2004; Pogozheva, Przydzial et al. 2005) Like rhodopsin, opioid receptors belong to class A of the GPCR superfamily. The crystallographic structure of the active state of rhodopsin is now available (Choe, Kim et al. 2011) and can be contrasted with the large body of literature that suggests a common active conformation among the class-A GPCRs. (Karnik, Gogonea et al. 2003) In the activated state TM6 undergoes outward rigid-body translation toward TM5, but away from TM3 and TM7. As a result, a cavity opens up in the intracellular domain in contact with G-proteins. Similar movements have been also suggested for TM1-3 and TM7.(Lin and Sakmar 1996; Gether, Lin et al. 1997; Altenbach, Cai et al. 2001) A better understanding of these activation mechanisms at the molecular level could lead to new drugs geared towards the therapeutic regulation of their functions.

Decaillot FM et al. applied mutagenesis to study the mechanism of activation of the human δ-opioid receptor.(Decaillot, Befort et al. 2003) By analyzing 30 constitutively active mutants of this receptor, mutations hypothesized to produce distinct active conformations were grouped into four abutting areas of the receptor from the extracellular (group I) to the intracellular (group IV) domain. Details about the residues that form each group can be found in Decaillot FM. A sequential binding mechanism was proposed to activate the receptor.(Decaillot, Befort et al. 2003) Sequential binding in GPCRs is not uncommon. A similar mechanism has been postulated for the β2-adrenergic receptors.(Swaminath, Xiang et al. 2004) In the case of the δ-opioid receptor agonists bind to residues in group I comprising a hydrophobic region in EL3, weakening interactions with TM6 and TM7 in the extracellular domain thus initiating a signal. Next, the ligand enters the binding pocket disrupting interactions in groups II and III. Group II residues form a molecular switch that controls movements of TM3. Group III residues are closest to the binding site, consist of patches of hydrophilic and hydrophobic residues and form a network of interactions between residues derived from TM3, 6 and 7. The disruption of these interactions results in a receptor state that is susceptible to activation and helps propagate signals to the intracellular side. It was hypothesized that the amphiphilic nature of opiates and opioid ligands makes them complementary to residues in group III, i.e., the hydrophilic portion of

the ligands disrupt the hydrogen-bonding network, while the hydrophobic portion compete with the hydrophobic residues. Finally, disrupting the interactions in group 4 residues results in the separation of TM6 and TM7 in the intracellular side and possibly destabilizing interactions with Gα and exposure to other secondary protein effectors.

Insights about the conformation of the activated state of the μ-opioid receptor are based on modeling experimental distance constraints derived from site-directed mutagenesis, inter-helix H-bonds, disulphide bonds, and engineered Zn^{2+} binding sites between the μ-opioid receptor and analogues of the receptor-bound conformation of a cyclic tetra-peptidomimetic, JOM6.(Fowler, Pogozheva et al. 2004) Structural data for the active state were also derived from disulphide bonds between TM5 and TM6 in the ACM3 muscarinic receptor,(Ward SD, JBC 2002) intrinsic allosteric Zn^{2+} binding sites in TM5 and TM6 of the β_2-adrenergic receptor,(Swaminath, Lee et al. 2003) engineered activating metal-coordination center akin to those between TM3 and TM7 in the β_2-adrenergic(Elling, Thirstrup et al. 1999) and tachykinin(Holst, Elling et al. 2000) receptors, between TM2 and TM3 of the MC4 melanocortin receptor. Finally one hydrogen bond constraint from the δ-opioid receptor(Decaillot, Befort et al. 2003) was introduced. A comparison between the modeled structures of the active and inactive states of the μ-opioid receptor is shown in Figure 1. A noticeable difference is seen in TM6 highlighting the rigid-body movement described for the δ-opioid receptor.

2.3 Internalization of opioid receptors and changes in downstream signaling

Signal transduction by the μ-opioid receptor is determined by properties of the ligand such as affinity, potency, efficacy, bio-availability and half-life, collectively defined as 'relative activity' or RA.(Martini and Whistler 2007) In addition, the length of time the receptor-ligand complex remains coupled to the G-protein, is controlled by receptor desensitization, endocytosis and to an extent the pharmacokinetic properties of the ligand. It has been noted that ligand activity and endocytosis do not have a linear relationship.(Martini and Whistler 2007) Hence, an interplay of relative activity versus endocytosis (RAVE) for each ligand determines the magnitude of the signal transduced. Thus each ligand-receptor complex has an associated RAVE value. As highlighted by Martini L et al.(Martini and Whistler 2007) endogenous peptides have good RA values at the μ-opioid receptor, and also induce significant desensitization and endocytosis. Based on this reasoning, the good balance between their RA and VE values explain why they do not induce tolerance. Another example is methadone. Methadone has comparable potency with encephalin and is also an equally good receptor internalizer.(Whistler, Chuang et al. 1999) Nonetheless, it has a longer half-life compared to other opioids, and consequently a larger RA value giving rise to a moderately higher RAVE value. The extension of the RAVE analysis to morphine is more complicated and invokes secondary protein effectors and region-selective differences in receptor endocytosis.(Martini and Whistler 2007) In general, agonists such as morphine with high RAVE values are more likely to induce tolerance. It has been demonstrated that the development of μ-opioid tolerance is inversely related to the ability of an agonist to promote receptor endocytosis or internalization.(Whistler, Chuang et al. 1999; Finn and Whistler 2001) This theory distinguishes two types of agonists based on their ability to stabilize different receptor conformational states, resulting in phosphorylation by different kinases. Depending on the type of kinase the receptor can be rapidly endocytosed, resensitized and recycled to the cell surface, preventing the development of tolerance.

The formation of an opioid ligand-receptor complex results in structural changes at the extracellular and transmembrane domains, which are propagated to the intracellular

domain followed by the dissociation of G-proteins; phosphorylation by G-protein coupled receptor kinases (GRK), protein kinase A (PKA) and C (PKC); and binding by other proteins such as β-arrestins.(Eguchi 2004) The phosphorylated receptor is endocytosed, whereby it is re-sensitized and recycled to the cell surface or it is marked for degradation. Unlike PKA and PKC, specific GRK-phosphorylation triggers the recruitment of β-arrestins, receptor internalization, resensitization and recycling to the cell surface. This dynamic recycling process has been suggested as crucial to circumvent development to drug tolerance. Tolerance-causing agonists impede receptor endocytosis and/or resensitization, while non-tolerance-inducing drugs promote rapid receptor desensitization-internalization-resensitization and recycling.(Martini and Whistler 2007)

The exact cause of development of tolerance is still a subject of debate. Nonetheless, it is generally accepted that chronic administration of opiates for analgesia gives rise to tolerance. The cellular mechanism of tolerance may involve downstream compensatory changes in neuronal circuits.(Eguchi 2004) The continual and sustained inhibition of adenylate cyclase activity triggers a positive feedback to compensate for the low intracellular levels of cAMP, resulting in the reversible superactivation of adenylate cyclase. This up-regulation of enzyme activity restores the cellular concentration of cAMP, resulting in cells being tolerant to the opiate and also dependent on it given that withdrawing the drug or introducing an antagonist gives rise to abnormally high levels of cAMP and also a restoration of the normal activity level of adenylate cyclase.(Sharma, Klee et al. 1975) The change is delayed but relatively stable and is known to be responsible for opiate tolerance and dependence.(Sharma, Klee et al. 1975) The combined inhibition and up-regulation of adenylyl cyclase provide a means of activating and deactivating neuronal circuits and may play a role in a memory process. It was later shown that the adenylate cyclase V and Gβγ played a role in this activation.(AvidorReiss, Nevo et al. 1996)

2.4 Point-mutation studies to identify key residue targets for phosphorylation

Mutation studies have been successful in identifying key cytosolic domains and residues of ligand-activated μ-opioid receptors, which are liable to phosphorylation, and potentially directly involved in agonist-dependent receptor internalization. (Celver, Lowe et al. 2001; El Kouhen, Burd et al. 2001; Celver, Xu et al. 2004) Truncation of the μ-opioid receptor at Ser363 produced a mutant that was not phosphorylated, and was endocytosed and recycled more slowly than the wild-type,(Qiu, Law et al. 2003) suggesting that phosphorylating residues in this segment may be important for internalization. Cleaving off the entire C-terminal resulted in increased agonist-independent internalization and recycling,(Waldhoer, Bartlett et al. 2004) indicating a greater exposure of some residues critical for the dynamic recycling machinery. Utilizing a single agonist, [D-Ala^2,MePhe^4,Gly^5-*ol*]enkephalin (DAMGO), the mutation of Thr180 to alanine in the second intracellular loop prevented receptor desensitization, while alanine scanning of serine or threonine in the third cytoplasmic loop did not inhibit receptor desensitization.(Celver, Lowe et al. 2001) In a DAMGO-induced receptor activation study, mutations of C-terminal serine/threonine residues identified three phosphorylation sites: Ser363, Thr370 and Ser375. The S375A mutant decreased the rate of receptor internalization, while the S363A and T370A double mutant accelerated the rate of internalization,(El Kouhen, Burd et al. 2001) which may suggest that the combined phosphorylation of Ser363 and Thr370 attenuates receptor internalization. Other studies employing etorphine and multiple mutations have also identified Ser356 and Ser363,(Burd, El-Kouhen et al. 1998) and Thr394 (using DAMGO)(Pak, Odowd et al. 1997; Wolf, Koch et al. 1999) as sites for phosphorylation that

result in down-regulation of the μ-opioid receptor. Mutation of Ser356 and Ser363 simultaneously did not alter receptor phosphorylation, but the mutations prevented down-regulation of the receptor suggesting that the absence of down-regulation was not due to the removal of phosphorylation sites. Down-regulation may be occurring through a phosphorylation-independent mechanism or these two sites are not phosphorylated. This is contrary to later studies that demonstrated that Ser363 is phosphorylated.(El Kouhen, Burd et al. 2001) The T394A mutant is more rapidly internalized and resensitized relative to the wild-type μ-opioid receptor. These mutation studies show that multiple phosphorylation motifs may be needed for internalization and that not every phosphorylation site is phosphorylated.

3. Discovery and development of opioid receptor ligands

3.1 Endogenous opioid ligands

Extensive structural and pharmacological studies have been performed to understand the mechanisms of action of opioids as well as for the design of new and more efficient opioid-based painkillers. The opioid agonists propagate their analgesic effects by interacting with opioid receptors. They are both endogenously expressed peptides and exogenous opiates. The term opiate is reserved for foreign substances introduced into the body to target opioid receptors. The endogenous peptides enkephalins, dynorphins, β-endorphins and nociceptins are excised from their precursors pro-enkephalin, pro-dynorphin, pro-opiomelanocortin and pro-nociceptin/orphanin FQ, respectively. The majority of these peptides comprise a conserved N-terminal YGGF motif,(Gentilucci, Squassabia et al. 2007) except the uncharacteristically short peptides endomorphin-1 (YPWF-NH_2) and endomorphin-2 (YPFF-NH_2) that are considered analogues of the YGGF motif. A list of endogenous peptides, their precursors and receptor selectivity is presented in Table 1.

Peptide	Sequences	Precursor	Selectivity
Endomorphin-1	YPWF-NH_2	ND[a]	μ
Endomorphin-2	YPFF-NH_2		
β-endorphin	**YGGF**MTSEKSQTPLVTLFK NAIIKNAYKKGE	Pro-opiomelanocortin	μ=δ
[Leu^5]enkephalin	**YGGF**L	Pro-enkephalin	δ
[Met^5]enkephalin	**YGGF**M		
Metorphinamide	**YGGF**MRRV-NH_2		
Deltorphin A	YmFHLMD-NH_2	ND[a]	δ
Deltorphin I	YaFDVVG-NH_2		
Deltorphin II	YaFEVVG-NH_2		
Dynorphin A	**YGGF**LRRIRPKLKWDNQ	Pro-dynorphin	κ
Dynorphin A(1-8)	**YGGF**LRRI		
Dynorphin B	**YGGF**LRRQFKVVT		
α-neoendorphin	**YGGF**LRKYPK		
β-neoendorphin	**YGGF**LRKYP		
Nociceptin	FGGFTGARKSARKLANQ	Pro-nociceptin / Orphanin FQ	ORL-1[b]

[a] Not yet determined. The conserved YGGF sequence is shown in bold
[b] Orphan opioid receptor-like 1

Table 1. Endogenous opioid peptides, the precursor and receptor selectivity.

Endomorphin-1 and endomorphin-2 are highly potent, selective μ-opioid receptor endogenous peptides isolated from mammals, and elicit responses similar to that of morphine.(Zadina, Hackler et al. 1997; Horvath 2000) The endogenous peptides are advantageous in that they do not display any of the side effects of opiates (see below); however, they are not effective in clinical settings because of *in vivo* degradation by peptidases.(Witt, Gillespie et al. 2001) Notwithstanding their degradation, these peptides and their analogues have been utilized extensively as tools to probe receptor categorization and structure-activity relationships.(Hruby and Agnes 1999; Gentilucci, Squassabia et al. 2007) The exogenous opiates on the other hand are more effective in pain management, but present numerous undesirable side effects, some of which are highlighted below. As such, several efforts are being undertaken to identify beneficial analgesics with minimal to no side effects.

3.2 Potent opioid-based analgesics

Interests in identifying more effective analgesics have led to the reporting of a large number potent opioid peptide and non-peptide compounds that are generally classified as agonists or antagonists.(Pan 1998; Stevens, Jones et al. 2000; Eguchi 2004; Waldhoer, Bartlett et al. 2004; Gentilucci, Squassabia et al. 2007; Prisinzano and Rothman 2008; Volpe, Tobin et al. 2011) In spite of the multitude of known opioid compounds, only a relatively small number has been approved for clinical use. The majority of these prescribed analgesics are relatively selective for the μ-opioid receptor,(Volpe, Tobin et al. 2011) though at sufficiently higher doses interactions with the other opioid receptors will occur. While some of these compounds are selective for either the μ (morphine), κ (salvinorin A), or δ (naltrindole) opioid receptors, some are non-selective and display mixed agonist/antagonist responses, for example buprenorphine, pentazocine and butorphanol. Buprenorphine is a partial μ-agonist and partial κ-antagonist that is administered clinically for opioid detoxification and maintenance.(Blakeney, Reid et al. 2007)

Compound	Receptor	Function[a]	Compound	Receptor	Function[a]
Morphine*	M	A	Cyclazocine*	μ/κ	A/AN
Fentanyl*	"	"	Pentazocine*	"	"
Hydrocodone*	"	"	Nalbuphine*	"	"
Levorphanol*	"	"	SIOM	Δ	A
Meperidine*	"	"	SCN-80	"	"
Sufentanyl*	"	"	TAN-67	"	"
Methadone*	"	"	Ketocyclazocine	K	A
Oxycodone*	"	"	Ethyl Ketocyclazocine	"	"
Oxymorphone*	"	"	U-50,488	"	"
Codeine*	"	"	Salvinorin A	"	"
Naloxone*	"	AN	6′-GNTI[a]	"	"
Buprenorphine*	μ/κ	A/AN	5′-GNTI[a]	"	AN
Butorphanol*	"	"	Bremazocine	μ/δ/κ	A/AN

A = agonist; AN = antagonist
*Currently in clinical use. [a] GNTI: guanidino-naltrindole

Table 2. Opioid receptor ligands.

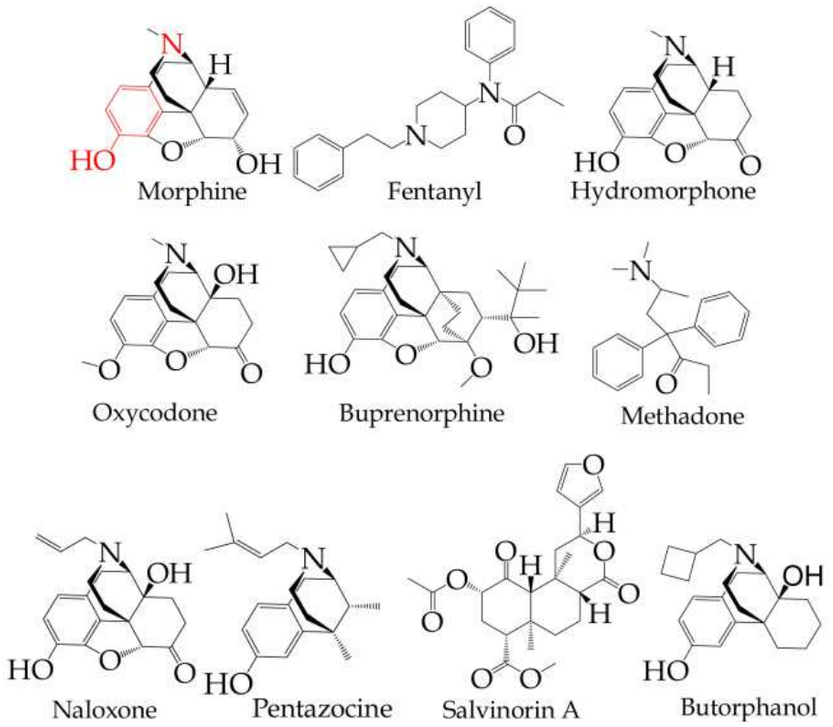


Fig. 2. The chemical structures of some exogenous opiates. The classical "message" tyramine moiety is colored in red in the structure of morphine.

The classification of some opioid compounds is given in Table 2. The chemical structures of selected compounds are shown in Figure 2. Several factors affect the potency of an analgesic, including route of administration, whether they act as full or partial agonists, ability to cross the blood-brain barrier (physico-chemical properties) and their effects on other major physiological systems.(Volpe, Tobin et al. 2011) Some potency comparisons with morphine worth mentioning include the following: fentanyl when administered intramuscularly is about 100 fold more potent; hydromorphone is 6-8 fold more potent;(Inturrisi 2002) and oral oxycodone is about 1.8 times more potent.(Curtis, Johnson et al. 1999) Though a partial agonist buprenorphine is reported to be 25-40 times more potent than morphine.(Blakeney, Reid et al. 2007)

3.3 Pharmacophoric features of opioid ligands

Numerous structure-activity relations (SAR) studies have been carried out on opioid receptor ligands to determine features that drive affinity or efficacy with the goal of generating more effective therapeutic compounds,(Eguchi 2004; Metcalf and Coop 2005; Prisinzano and Rothman 2008; Yongye, Appel et al. 2009, amongst others). SAR studies employing site-directed substitutions and constraints of endogenous peptides, as well as modifications of morphine have provided valuable insights about the pharmacophoric features, ligand selectivity and biological roles of opioid receptors.(Blakeney, Reid et al. 2007) For example it has been determined that a positively charged amine group, an aromatic moiety and a hydrophobic group result in tight binding of morphine. A salt-bridge is formed between the protonated amine and an aspartate residue in TM3, π-π

stacking interactions between the aromatic group and residues in the binding pocket and hydrophobic-hydrophobic interactions. In endogenous peptides the N-terminal tyrosine contains a protonated amine and aromatic group, akin to the aromatic ring (A) and basic nitrogen (N) in morphine, Figure 3. This moiety termed tyramine is common to a majority of opioids, though there are some notable potent and selective opiates that lack this classical pharmacophore: Salvinorin A was the first highly potent, non-nitrogen opiate agonist selective towards the κ-opioid receptor;(Roth, Baner et al. 2002) one of its analogues, herkinorin became the first non-nitrogenous agonist selective towards the μ-opioid receptor.(Harding, Tidgewell et al. 2005) Furthermore, the phenylalanine side chain in endogenous peptides mimics the hydrophobic feature (B) of morphine (ring C). It should be pointed out that due to size differences between the peptides and morphine, the interactions between their respective hydrophobic features (B) and the receptor are different.

The observation of the occurrence of a common structural feature amongst opioid ligands gave rise to the "message-address" concept of ligand-receptor interactions, i.e., the same message (signal transduction) is delivered to different addresses (receptors). For the endogenous peptides the message comprises the conserved YGGF motif, with the exceptions cited in Table 1, while for the opiates the tyramine moiety represents the message. The other varied segments of the ligands make up the address and confer selectivity.

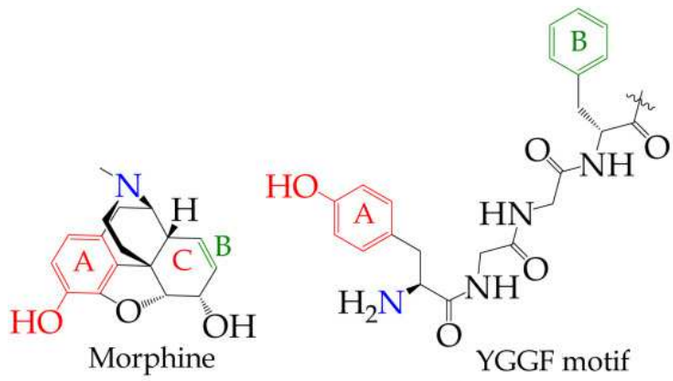


Fig. 3. Chemical structures of morphine and the truncated "message" motif of an endogenous peptide. The YGGF represents amino acids: Y, tyrosine; G, glycine and F, phenylalanine.

Generating pharmacophore models for opioid receptors have followed two traditional approaches: ligand-based or docking-based. Ligand-based methods involve identifying and superimposing common substructures of low energy conformers from which features that drive biological activity are determined. However, because of the inherent difficulties of superimposing structurally different scaffolds these efforts have typically revolved around congeneric series. See Shim J et al.(Shim, Coop et al. 2011) and references therein. In ligand-based virtual screenings via multi-conformer ensembles, the quality and coverage of the conformational ensemble are important. The production of the conformers can be computationally intensive, especially for compounds with a large number of rotatable bonds. Thus, reducing the size of multi-conformer databases and the number of query conformers, while simultaneously reproducing the bioactive conformer with good accuracy,

is of crucial interest. A recent protocol that takes into account these aspects has been proposed.(Yongye, Bender et al. 2010) This protocol and other important aspects of conformational coverage in ligand-based virtual screening methods have been recently revised.(Musafia and Senderowitz 2010) On the other hand, docking-based approaches are most valuable when experimental structures of receptors are available. The absence of experimental opioid receptor structures means docking-based methods must rely on homology models. Moreover, for docking-based virtual screening, one has to contend with no induced fit and the possibility of different binding modes.

The identification of enkephalins and δ-opioid receptors fueled interests in developing ligands that target this receptor. The observation that co-administration of δ-opioid receptor antagonists with μ-opioid receptor agonists produced analgesia without the side effect of μ-only agonists further served as motivation to identify δ-selective opioids. Hence, considerable efforts have been devoted to studying the SAR of δ-opioid receptor ligands using both pharmacophore and quantitative structure-activity relationship modeling. See Bernard D et al.(Bernard, Coop et al. 2007) and references therein. Employing the *conformationally sampled pharmacophore* (CSP) approach Bernard D et al. were able to differentiate between δ-opioid receptor agonists and antagonists.(Bernard, Coop et al. 2003; Bernard, Coop et al. 2005) An advantage of the CSP method is the inclusion of high energy conformers in describing pharmacophores, the justification stemming from the fact that ligands may bind in higher energy conformers stabilized by intermolecular interactions with receptors. The CSP methodology was later applied to peptide and nonpeptide agonists to derive pharmacophore models of δ-opioid receptor ligands.(Bernard, Coop et al. 2007) Three pharmacophore points were considered: aromatic (A), basic nitrogen (N) and hydrophobic group (B).

Utilizing efficacy as the activity index, CSP was extended to five peptides and twenty nonpeptides comprising μ-opioid receptor ligands, to derive an aggregate pharmacophore. By analyzing a diverse group of agonists, partial agonists and antagonists the following conclusions were derived: interactions with the B or hydrophobic site of oripavines (etorphine, buprenorphine and diprenorphine) modulated the degree of agonism; agonists with bulky B groups adopt a pose in which interactions occur with both the basic amine and the B site; agonists with large N-substituents are oriented such that the substituents occupy the position of the traditional B site. The resultant pharmacophore is an aromatic group (A), a basic amine (N), a hydrophobic group (B) and N-substituents (S). The investigators claim that such an approach would facilitate efforts to develop compounds that possess both μ-agonistic and δ-antagonistic properties even though the cell lines only expressed the μ-opioid receptor.(Shim, Coop et al. 2011) Furthermore, depending on the structural class of the ligand, N-subsituents can enhance agonism or antagonism. For example, *N*-allyl and *N*-cyclopropylmethyl substituents in etorphines give rise to better agonists compared to morphine,(Gorin and Marshall 1977) while they induce antagonism in 4,5-epoxymorphinans.(Shim, Coop et al. 2011)

The currently known κ-opioid receptor agonists have been classified into eight structural classes(Yamaotsu and Hirono 2011): peptides (dynorphins), benzomorphans (pentazocine), morphinans (butorphanol), arylacetamines (U-69593), diazabicyclononanones (HZ2), bicyclic guanidines (TPI-614-1), benzodiazepines (±tifluadom) and neocleorodane diterpenes (salvinorin A). A comprehensive review of these classes and the history of the development of κ-opioid receptor ligand pharmacophores was published recently by Yamaotsu N et al.(Yamaotsu and Hirono 2011) Evidently, the structural diversity of these

classes making it difficult to construct a consensus pharmacophore model. Previous SAR and pharmacophore analyses of κ-opioid receptor ligands are typically confined to structural analogues. Yamaotsu N et al. proposed a consensus pharmacophore encompassing all eight classes using seven compounds in both the training and test sets. Superposition was based on the physico-chemical properties of groups of atoms. The consensus pharmacophore comprised three hydrophobic groups, a hydrogen bond donor and three hydrogen bond acceptors. These pharmacophoric features were employed to describe four binding orientations of the different classes of ligands for the κ-opioid receptor. It remains to be determined how this consensus pharmacophore will perform in virtual screening: for example screening a database, requiring that a given number of features match, followed by biological evaluation of the top scoring compounds. Additionally, in the search of opioid receptor ligands, structure similarity(Martinez-Mayorga, Medina-Franco et al. 2008; Yongye, Appel et al. 2009) and chemoinformatic analyses(Medina-Franco, Martínez-Mayorga et al. 2009) have been employed to develop SAR and to characterize highly dense combinatorial libraries.

3.4 Identification of opioid receptor ligands

A large and growing body of literature has reported the identification of opioid receptor ligands. In particular, improvements in high-throughput chemical synthesis have made possible the rapid and efficient generation of molecules, giving rise to thousands or millions of compounds in combinatorial libraries. Advances in molecular biology have also enabled the evaluation of millions of individual compounds against a number of different biological targets via high-throughput screening (HTS). However, some high content assays, such as *in vivo* studies, are not amenable to the high-throughput miniaturization required to screen millions of individual compounds. In such cases, screening libraries using a mixture-based format(Houghten, Pinilla et al. 1999; Pinilla, Appel et al. 2003; Houghten, Dooley et al. 2006) (also known as positional scanning-synthetic combinatorial libraries or PS-SCL) enables the evaluation of thousands to millions of molecules in approximately a hundred to a few hundred samples. PS-SCL have been used to successfully identify active molecules for a variety of biological targets. (Houghten, Pinilla et al. 1999; Pinilla, Appel et al. 2003; Houghten, Pinilla et al. 2008) In the case of opioid receptors highly active peptides (Dooley, Chung et al. 1994; Houghten, Dooley et al. 2006) and peptidomimetics have been identified. (Houghten, Dooley et al. 2006) This technique has recently found new applications in the search of conotoxins (Armishaw, Singh et al.) and *in-vivo* screening (Reilley, Giulianotti et al.). A step forward in the development of peptides with therapeutic relevance corresponds to the formation of cyclic structures. Cyclic peptides are therapeutically attractive due to their high bioavailability, potential selectivity, and scaffold novelty. In addition, the presence of D-residues induces conformational preferences not followed by peptides consisting of only naturally abundant L-residues. Therefore, the development of synthetic schemes and comprehending how amino acids induce turns in peptides is significant in peptide design. For example, a successful method for the synthesis of cyclic peptides by the intramolecular aminolysis of peptide thioesters, has been recently reported,(Li, Yongye et al. 2009) and the corresponding explicit solvent molecular dynamics simulations were produced and analyzed.(Yongye, Li et al. 2009) The cyclic tetra-peptidomimetic, JOM6, (Fowler, Pogozheva et al. 2004) is an example of a conformationally constrained peptide that retains activity against the μ-opioid receptor. It is anticipated that research will continue in this direction.

The search of opioid receptor ligands using experimental screening of combinatorial libraries has been complemented using computational methods. *In silico* methods can be incorporated at different stages of the drug discovery process, from library design to lead optimization. (Brooijmans and Kuntz 2003) Computational methods are largely applied to corporate chemical collections (Bajorath 2002) as well as combinatorial chemical libraries. (Houghten, Pinilla et al. 2008) However, limited efforts have been reported so far to explicitly integrate information from mixture-based combinatorial libraries and computational techniques (López-Vallejo, Caulfield et al. 2011; Yongye, Pinilla et al. 2011). The structural analogy contained in combinatorial libraries in general and in mixture-based libraries in particular deserves particular considerations. Virtual screening may assist in downsizing large compound libraries and the selection of a smaller set of promising hits, whereas mixture-based screening may screen out some of the false positives of virtual screening. The integration of mixture-based combinatorial library screening data and virtual screening information has been undertaken. In the particular case of opioid receptors, the predicted activity obtained from the experimental mixture-based screening of a large library of bicyclic guanidines was combined with structural similarity methods. This approach allowed categorizing the molecules as actives, activity cliffs, diverse compounds and missed hits.(Yongye, Pinilla et al. 2011)

4. Conclusions

Ever since the discovery of opioid receptors as the principal mediators of analgesia and the identification of endogenous peptides as well as opiates that elicit analgesic responses, considerable efforts have been devoted to finding compounds that target these receptors with the aim of alleviating the sensation of pain. While the endogenous peptides do not display any side effects, their use in clinical settings is hampered because of *in vivo* degradation by protein-digesting enzymes. Opiates are more effective, but adverse side effects such as tolerance, dependence and addiction limit their prolonged usage; thus the continual search for more efficient analgesics. Several compounds have been reported as opioid receptor ligands, however, only a relatively few are currently prescribed in clinical settings with morphine being the prototypical μ-opioid agonist. A high proportion of opioid-based drugs is selective toward the μ-opioid receptor, and still retains untoward side effects prompting extensive studies about the molecular origins of these undesirable properties.

This review focuses on structural aspects of opioid receptors and opioid receptor ligands, with special emphasis on the μ-opioid receptor. The information presented here can be summarized as follows:

1. Considerable evidence now point to the existence of opioid receptors as homo- or hetero-oligomeric complexes and that their pharmacological responses may be cross-modulated. For example the co-administration of a μ-opioid agonist with a δ-opioid antagonist suppressed side effects such as dependence and tolerance while retaining μ-agonist induced analgesia. The realization of this potential for cross-modulation has generated interests in the development of bivalent ligands. The ligands may be individual compounds that possess mixed agonist/antagonist properties or a separate agonist and antagonist tethered through a linker. Future directions of research in analgesia will continue to point towards agonists with acceptable side effects, designing bivalent ligands, or ligands with mixed receptor specificities and functions.

2. While the exact mechanisms of development of tolerance are still under debate, the current models suggest a combination of ligand-induced conformational changes and receptor desensitization, as well as down-stream compensatory changes of secondary effectors.
3. Promising computational methods such as consensus pharmacophore models using different structural scaffold might serve a role in identifying ligands with mixed secondary functional profiles. Understanding the cross-talk between the different signaling pathways of the opioid receptors will also be significant.
4. Production and analysis of a large number of compounds with potential affinity to opioid receptors are possible. However, considerably more work will need to be done to understand and design compounds with high analgesic effect and lower side effects. To that end, a more detailed understanding of the signaling process upon opioid receptor activation is needed.

5. Acknowledgement

This work was supported by the State of Florida, Executive Officer of the Governor's Office of Tourism, Trade and Economic Development.

6. References

Abdelhamid, E. E., M. Sultana, *et al.* (1991). "Selective blockage of delta-opioid receptors prevents the development of morphine-tolerance and dependence in mice." Journal of Pharmacology and Experimental Therapeutics 258(1): 299-303.

Altenbach, C., K. W. Cai, *et al.* (2001). "Structure and function in rhodopsin: Mapping light-dependent changes in distance between residue 65 in helix TM1 and residues in the sequence 306-319 at the cytoplasmic end of helix TM7 and in helix H8." Biochemistry 40(51): 15483-15492.

Ananthan, S. (2006). "Opioid ligands with mixed m/d opioid receptor interactions: An emerging approach to novel analgesics." AAPS J. 8(1): E118-E125.

Armishaw, C. J., N. Singh, *et al.* (2010). "A synthetic combinatorial strategy for developing alpha-conotoxin analogs as potent alpha7 nicotinic acetylcholine receptor antagonists." J. Biol. Chem. 285: 1809-1821.

AvidorReiss, T., I. Nevo, *et al.* (1996). "Chronic opioid treatment induces adenylyl cyclase V superactivation - Involvement of G beta gamma." Journal of Biological Chemistry 271(35): 21309-21315.

Bajorath, J. (2002). "Integration of virtual and high-throughput screening." Nat. Rev. Drug Discov. 1(11): 882.

Balboni, G., S. Salvadori, *et al.* (2011). "Opioid bifunctional ligands from morphine and the opioid pharmacophore Dmt-Tic." European Journal of Medicinal Chemistry 46(2): 799-803.

Bernard, D., A. Coop, *et al.* (2003). "2D conformationally sampled pharmacophore: A ligand-based pharmacophore to differentiate delta opioid agonists from antagonists." Journal of the American Chemical Society 125(10): 3101-3107.

Bernard, D., A. Coop, *et al.* (2005). "Conformationally sampled pharmacophore for peptidic delta opioid ligands." Journal of Medicinal Chemistry 48(24): 7773-7780.

Bernard, D., A. Coop, *et al.* (2007). "Quantitative conformationally sampled pharmacophore for delta opioid ligands: Reevaluation of hydrophobic moieties essential for biological activity." Journal of Medicinal Chemistry 50(8): 1799-1809.

Blakeney, J. S., R. C. Reid, *et al.* (2007). "Nonpeptidic ligands for peptide-activated G protein-coupled receptors." Chemical Reviews 107(7): 2960-3041.

Botelho, A. V., N. J. Gibson, *et al.* (2002). "Conformational energetics of rhodopsin modulated by nonlamellar-forming lipids." Biochemistry 41(20): 6354-6368.

Botelho, A. V., T. Huber, *et al.* (2006). "Curvature and hydrophobic forces drive oligomerization and modulate activity of rhodopsin in membranes." Biophysical journal 91(12): 4464-4477.

Brooijmans, N. and I. D. Kuntz (2003). "Molecular recognition and docking algorithms." Annu Rev Biophys Biomol Struct. 32: 335-373.

Burd, A. L., R. El-Kouhen, *et al.* (1998). "Identification of serine 356 and serine 363 as the amino acids involved in etorphine-induced down-regulation of the mu-opioid receptor." Journal of Biological Chemistry 273(51): 34488-34495.

Celver, J., M. Xu, *et al.* (2004). "Distinct domains of the mu-opioid receptor control uncoupling and internalization." Molecular Pharmacology 65(3): 528-537.

Celver, J. P., J. Lowe, *et al.* (2001). "Threonine 180 is required for G-protein-coupled receptor kinase 3- and beta-arrestin 2-mediated desensitization of the mu-opioid receptor in Xenopus oocytes." Journal of Biological Chemistry 276(7): 4894-4900.

Choe, H. W., Y. J. Kim, *et al.* (2011). "Crystal structure of metarhodopsin II." Nature 471(7340): 651-U137.

Cowan, A., X. Z. Zhu, *et al.* (1988). "Direct dependence studies in rats with agents selective for different types of opioid receptor." Journal of Pharmacology and Experimental Therapeutics 246(3): 950-955.

Curtis, G. B., G. H. Johnson, *et al.* (1999). "Relative potency of controlled-release oxycodone and controlled-release morphine in a postoperative pain model." European Journal of Clinical Pharmacology 55(6): 425-429.

Decaillot, F. M., K. Befort, *et al.* (2003). "Opioid receptor random mutagenesis reveals a mechanism for G protein-coupled receptor activation." Nature Structural Biology 10(8): 629-636.

Dietis, N., R. Guerrini, *et al.* (2009). "Simultaneous targeting of multiple opioid receptors: a strategy to improve side-effect profile." British Journal of Anaesthesia 103(1): 38-49.

Dooley, C. T., N. N. Chung, *et al.* (1994). "An all D-amino-acid opioid peptide with central analgesic activity from a combinatorial library." Science 266(5193): 2019-2022.

Eguchi, M. (2004). "Recent advances in selective opioid receptor agonists and antagonists." Medicinal Research Reviews 24(2): 182-212.

El Kouhen, R., A. L. Burd, *et al.* (2001). "Phosphorylation of Ser363, Thr370 and Ser375 residues within the carboxyl tail differentially regulates μ-opioid receptor internalization." Journal of Biological Chemistry 276(16): 12774-12780.

Elling, C. E., K. Thirstrup, *et al.* (1999). "Conversion of agonist site to metal-ion chelator site in the beta(2)-adrenergic receptor." Proceedings of the National Academy of Sciences of the United States of America 96(22): 12322-12327.

Fanelli, F. and P. G. De Benedetti (2006). "Inactive and active states and supramolecular organization of GPCRs: insights from computational modeling." Journal of Computer-Aided Molecular Design 20(7-8): 449-461.

Finn, A. K. and J. L. Whistler (2001). "Endocytosis of the mu opioid receptor reduces tolerance and a cellular hallmark of opiate withdrawal." Neuron 32(5): 829-839.

Fowler, C. B., I. D. Pogozheva, *et al.* (2004). "Refinement of a homology model of the μ-opioid receptor using distance constraints from intrinsic and engineered zinc-binding sites." Biochemistry 43: 8700-8710.

Fowler, C. B., I. D. Pogozheva, *et al.* (2004). "Complex of an active μ-opioid receptor with a cyclic peptide agonist modeled from experimental constraints." Biochemistry 43: 15796-15810.

Gentilucci, L., F. Squassabia, *et al.* (2007). "Re-discussion of the importance of ionic interactions in stabilizing ligand-opioid receptor complex and in activating signal transduction." Current Drug Targets 8(1): 185-196.

Gether, U. (2000). "Uncovering molecular mechanisms involved in activation of G protein-coupled receptors." Endocrine Reviews 21(1): 90-113.

Gether, U., S. Lin, *et al.* (1997). "Agonists induce conformational changes in transmembrane domains III and VI of the beta(2) adrenoceptor." Embo Journal 16(22): 6737-6747.

Gorin, F. A. and G. R. Marshall (1977). "Proposal for biologically-active conformation of opiates and enkephalin." Proceedings of the National Academy of Sciences of the United States of America 74(11): 5179-5183.

Grossfield, A., M. C. Pitman, *et al.* (2008). "Internal Hydration Increases during Activation of the G-Protein-Coupled Receptor Rhodopsin." J. Mol. Biol. 381(2): 478-486.

Harding, W. W., K. Tidgewell, *et al.* (2005). "Neoclerodane diterpenes as a novel scaffold for mu opioid receptor ligands." Journal of Medicinal Chemistry 48(15): 4765-4771.

Holst, B., C. E. Elling, *et al.* (2000). "Partial agonism through a zinc-ion switch constructed between transmembrane domains III and VII in the tachykinin NK1 receptor." Molecular Pharmacology 58(2): 263-270.

Horvath, G. (2000). "Endomorphin-1 and endomorphin-2: pharmacology of the selective endogenous mu-opioid receptor agonists." Pharmacology & Therapeutics 88(3): 437-463.

Houghten, R. A., C. T. Dooley, *et al.* (2006). "In vitro and direct in vivo testing of mixture-based combinatorial libraries for the identification of highly active and specific opiate ligands." Aaps Journal 8(2): E371-E382.

Houghten, R. A., C. Pinilla, *et al.* (1999). "Mixture-based synthetic combinatorial libraries." J. Med. Chem. 42(19): 3743-3778.

Houghten, R. A., C. Pinilla, *et al.* (2008). "Strategies for the use of mixture-based synthetic combinatorial libraries: Scaffold ranking, direct testing, in vivo, and enhanced deconvolution by computational methods." J. Comb. Chem. 10(1): 3-19.

Hruby, V. and R. S. Agnes (1999). "Conformation-activity relationships of opioid peptides with selective activities at opioid receptors." Biopolymers 51: 391-410.

Hubbell, W. L., C. Altenbach, *et al.* (2003). "Rhodopsin structure, dynamics, and activation: A perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking." Membrane Proteins 63: 243-290.

Inturrisi, C. (2002). "Clinical pharmacology of opioids for pain." Clinical Journal of Pain 18: S3-S13.

Jaakola, V. P., M. T. Griffith, *et al.* (2008). "The 2.6 Angstrom Crystal Structure of a Human A(2A) Adenosine Receptor Bound to an Antagonist." Science 322(5905): 1211-1217.

Karnik, S. S., C. Gogonea, *et al.* (2003). "Activation of G-protein-coupled receptors: a common molecular mechanism." Trends in Endocrinology and Metabolism 14(9): 431-437.

Knapp, R. J., E. Malatynska, *et al.* (1995). "Molecular-biology and pharmacology of cloned opioid receptors." Faseb Journal 9(7): 516-525.

Kobilka, B. and G. F. X. Schertler (2008). "New G-protein-coupled receptor crystal structures: insights and limitations." Trends in Pharmacological Sciences 29(2): 79-83.

Kolinski, M. and S. Filipek (2008). "Molecular dynamics of μ opioid receptor complexes with agonists and antagonists." The Open Structural Biology Journal 2: 8-20.

Lahti, R. A., M. M. Mickelson, *et al.* (1985). "[^{3}H]U-69593 a highly selective ligand for the opioid κ-receptor." European Journal of Pharmacology 109(2): 281-284.

Lau, P. W., A. Grossfield, *et al.* (2007). "Dynamic structure of retinylidene ligand of rhodopsin probed by molecular simulations." Journal of Molecular Biology 372(4): 906-917.

Leighton, G. E., M. A. Johnson, *et al.* (1987). "Pharmacological profile of PD-117302, a selective kappa-opioid agonist." British Journal of Pharmacology 92(4): 915-922.

Li, J. H., S. J. Han, *et al.* (2007). "Distinct structural changes in a g protein-coupled receptor caused by different classes of agonist ligands." Journal of Biological Chemistry 282(36): 26284-26293.

Li, Y., A. Yongye, *et al.* (2009). "Synthesis of Cyclic Peptides through Direct Aminolysis of Peptide Thioesters Catalyzed by Imidazole in Aqueous Organic Solutions." Journal of Combinatorial Chemistry 11(6): 1066-1072.

Lin, S. W. and T. P. Sakmar (1996). "Specific tryptophan UV-absorbance changes are probes of the transition of rhodopsin to its active state." Biochemistry 35(34): 11149-11159.

López-Vallejo, F., T. Caulfield, *et al.* (2011). "Integrating virtual screening and combinatorial chemistry for accelerated drug discovery." Comb. Chem. High Throughput Screening 14(6): 475-487.

Maldonado, R., S. Negus, *et al.* (1992). "Precipitation of morphine-withdrawal syndrome in rats by administration of mu-selective, delta-selective and kappa-selective opioid antagonists." Neuropharmacology 31(12): 1231-1241.

Mansour, A., H. Khachaturian, *et al.* (1988). "Anatomy of CNS opioid receptors." Trends in Neuroscience 11: 308.

Martinez-Mayorga, K., J. L. Medina-Franco, *et al.* (2008). "Conformation-opioid activity relationships of bicyclic guanidines from 3D similarity analysis." Bioorg. & Med. Chem. 16(11): 5932-5938.

Martinez-Mayorga, K., M. C. Pitman, *et al.* (2006). "Retinal counterion switch mechanism in vision evaluated by molecular simulations." J. Am. Chem. Soc. 128: 16502-16503.

Martini, L. and J. L. Whistler (2007). "The role of mu opioid receptor desensitization and endocytosis in morphine tolerance and dependence." Current Opinion in Neurobiology 17(5): 556-564.

Medina-Franco, J. L., K. Martínez-Mayorga, *et al.* (2009). "Characterization of Activity Landscapes Using 2D and 3D Similarity Methods: Consensus Activity Cliffs." J. Chem. Inf. Model. 49(2): 477-491.

Meng, E. C. and H. R. Bourne (2001). "Receptor activation: what does the rhodopsin structure tell us?" Trends in Pharmacological Sciences 22(11): 587-593.

Metcalf, M. D. and A. Coop (2005). "Kappa opioid antagonists: Past successes and future prospects." Aaps Journal 7(3): E704-E722.

Musafia, B. and H. Senderowitz (2010). "Biasing conformational ensembles towards bioactive-like conformers for ligand-based drug design." Expert Opinion on Drug Discovery 5(10): 943-959.

Pak, Y., B. F. Odowd, *et al.* (1997). "Agonist-induced desensitization of the mu opioid receptor is determined by threonine 394 preceded by acidic amino acids in the COOH-terminal tail." Journal of Biological Chemistry 272(40): 24961-24965.

Palczewski, K., T. Kumasaka, *et al.* (2000). "Crystal structure of rhodopsin: A G protein-coupled receptor." Science 289(5480): 739-745.

Pan, Z. Z. (1998). "mu-opposing actions of the kappa-opioid receptor." Trends in Pharmacological Sciences 19(3): 94-98.

Parnot, C., S. Miserey-Lenkei, *et al.* (2002). "Lessons from constitutively active mutants of G protein-coupled receptors." Trends in Endocrinology and Metabolism 13(8): 336-343.

Pasternak, G. W. (1993). "Pharmacological mechanisms of opioid analgesics." Clinical Neuropharmacology 16(1): 1-18.

Pert, C. B., G. Pasternak, *et al.* (1973). "Opiate agonists and antagonist discriminated by receptor binding in brain." Science 182(4119): 1359-1361.

Pinilla, C., J. R. Appel, *et al.* (2003). "Advances in the use of synthetic combinatorial chemistry: mixture-based libraries." Nat. Med. 9(1): 118-122.

Pogozheva, I. D., A. L. Lomize, *et al.* (1998). "Opioid receptor three-dimensional structures from distance geometry calculations with hydrogen bonding constraints." Biophysical Journal 75: 612-634.

Pogozheva, I. D., M. J. Przydzial, *et al.* (2005). "Homology modeling of opioid receptor-ligand complexes using experimental constraints." The AAAPS Journal 7(2): E434-E448.

Prisinzano, T. E. and R. B. Rothman (2008). "Salvinorin A analogs as probes in opiold pharmacology." Chemical Reviews 108(5): 1732-1743.

Qiu, Y., P. Y. Law, *et al.* (2003). "mu-opioid receptor desensitization - Role of receptor phosphorylation, internalization, and resensitization." Journal of Biological Chemistry 278(38): 36733-36739.

Reilley, K., M. A. Giulianotti, *et al.* (2010). "Identification of Two Novel, Potent, Low-Liability Antinociceptive Compounds from the Direct In Vivo Screening of a Large Mixture-Based Combinatorial Library." AAAPS J. 12: 318-329.

Reisine, T. and G. Pasternak (1996). Opioid analgesics and antagonists, In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. Hardman J.G., Limbird L.E., pp 521-555, McGraw-Hill, New York.

Ridge, K. D. and K. Palczewski (2007). "Visual rhodopsin sees the light: Structure and mechanism of G protein signaling." Journal of Biological Chemistry 282(13): 9297-9301.

Roth, B. L., K. Baner, *et al.* (2002). "Salvinorin A: A potent naturally occurring nonnitrogenous kappa opioid selective agonist." Proceedings of the National Academy of Sciences of the United States of America 99(18): 11934-11939.

Rutherford, J. M., J. Wang, *et al.* (2008). "Evidence for a mu-delta opioid receptor complex in CHO cells co-expressing mu and delta opioid peptide receptors." Peptides 29(8): 1424-1431.

Schmauss, C. and T. L. Yaksh (1984). "*In vivo* studies on spinal opiate receptor systems mediating antinociception. 2. Pharmacological profiles suggesting a differential association of mu-receptor, delta-receptor and kappa-receptor with visceral chemical and cutaneous thermal stimuli in the rat." Journal of Pharmacology and Experimental Therapeutics 228(1): 1-12.

Sharma, S. K., W. A. Klee, *et al.* (1975). "Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance." Proceedings of the National Academy of Sciences of the United States of America 72(8): 3092-3096.

Shim, J., A. Coop, *et al.* (2011). "Consensus 3D Model of mu-Opioid Receptor Ligand Efficacy Based on a Quantitative Conformationally Sampled Pharmacophore." Journal of Physical Chemistry B 115(22): 7487-7496.

Stevens, W. C., R. M. Jones, *et al.* (2000). "Potent and Selective Indolomorphinan Antagonists of the Kappa-Opioid Receptor." J. Med. Chem. 43(14): 2759-2769.

Struts, A. V., G. F. J. Salgado, *et al.* (2011). "Retinal dynamics underlie its switch from inverse agonist to agonist during rhodopsin activation." Nature Structural & Molecular Biology 18(3): 392-394.

Swaminath, G., T. W. Lee, *et al.* (2003). "Identification of an allosteric binding site for ZN(2+) on the beta(2) adrenergic receptor." Journal of Biological Chemistry 278(1): 352-356.

Swaminath, G., Y. Xiang, *et al.* (2004). "Sequential binding of agonists to the beta(2) adrenoceptor - Kinetic evidence for intermediate conformational states." Journal of Biological Chemistry 279(1): 686-691.

Volpe, D. A., G. A. M. Tobin, *et al.* (2011). "Uniform assessment and ranking of opioid Mu receptor binding constants for selected opioid drugs." Regulatory Toxicology and Pharmacology 59(3): 385-390.

von Voigtlander, P. F., R. A. Lahti, *et al.* (1983). "U-50,488: a selective and structurally novel non-*mu*-(*kappa*)-opioid agonist." Journal of Pharmacology and Experimental Therapeutics 224(1): 7-12.

Waldhoer, M., S. E. Bartlett, *et al.* (2004). "Opioid receptors." Annual Review of Biochemistry 73: 953-990.

Waldhoer, M., J. Fong, *et al.* (2005). "A heterodimer-selective agonist shows in vivo relevance of G protein-coupled receptor dimers." Proceedings of the National Academy of Sciences of the United States of America 102(25): 9050-9055.

Whistler, J. L., H. H. Chuang, *et al.* (1999). "Functional dissociation of mu opioid receptor signaling and endocytosis: Implications for the biology of opiate tolerance and addiction." Neuron 23(4): 737-746.

Witt, K. A., T. J. Gillespie, *et al.* (2001). "Peptide drug modifications to enhance bioavailability and blood-brain barrier permeability." Peptides 22(12): 2329-2343.

Wolf, R., T. Koch, *et al.* (1999). "Replacement of threonine 394 by alanine facilitates internalization and resensitization of the rat mu opioid receptor." Molecular Pharmacology 55(2): 263-268.

Wollemann, M., S. Benyhe, *et al.* (1993). "The kappa-opioid receptor: evidence for the different subtypes." Life Sciences 52(7): 599-611.

Xu, W., A. Sanz, *et al.* (2008). "Activation of the mu opioid receptor involves conformational rearrangements of multiple transmembrane domains." Biochemistry 47(40): 10576-10586.

Yamaotsu, N. and S. Hirono (2011). "3D-pharmacophore identification for κ-opioid agonists using ligand-based drug-design techniques." Topics in Current Chemistry 299: 277-307.

Yongye, A. B., J. R. Appel, *et al.* (2009). "Identification, structure-activity relationships and molecular modeling of potent triamine and piperazine opioid ligands." Bioorg. & Med. Chem. 17(15): 5583-5597.

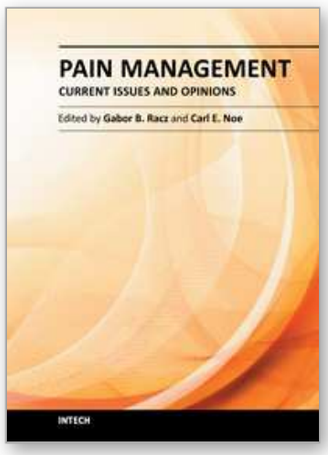
Yongye, A. B., A. Bender, *et al.* (2010). "Dynamic clustering threshold reduces conformer ensemble size while maintaining a biologically relevant ensemble." J. Comput. Aided. Mol. Des. 24: 675-686.

Yongye, A. B., Y. M. Li, *et al.* (2009). "Modeling of peptides containing D-amino acids: implications on cyclization." J. Comp.-Aid. Mol. Des. 23(9): 677-689.

Yongye, A. B., C. Pinilla, *et al.* (2011). "Integrating computational and mixture-based screening of combinatorial libraries." Journal of Molecular Modeling 17: 1473-1482.

Zadina, J. E., L. Hackler, *et al.* (1997). "A potent and selective endogenous agonist for the mu-opiate receptor." Nature 386(6624): 499-502.

Zhu, Y. X., M. A. King, *et al.* (1999). "Retention of supraspinal delta-like analgesia and loss of morphine tolerance in delta opioid receptor knockout mice." Neuron 24(1): 243-252.



Pain Management - Current Issues and Opinions

Edited by Dr. Gabor Racz

ISBN 978-953-307-813-7

Hard cover, 554 pages

Publisher InTech

Published online 18, January, 2012

Published in print edition January, 2012

Pain Management - Current Issues and Opinions is written by international experts who cover a number of topics about current pain management problems, and gives the reader a glimpse into the future of pain treatment. Several chapters report original research, while others summarize clinical information with specific treatment options. The international mix of authors reflects the "casting of a broad net" to recruit authors on the cutting edge of their area of interest. Pain Management - Current Issues and Opinions is a must read for the up-to-date pain clinician.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Austin B. Yongye and Karina Martînez-Mayorga (2012). Molecular Aspects of Opioid Receptors and Opioid Receptor Painkillers, Pain Management - Current Issues and Opinions, Dr. Gabor Racz (Ed.), ISBN: 978-953-307-813-7, InTech, Available from: http://www.intechopen.com/books/pain-management-current-issues-and-opinions/molecular-aspects-of-opioid-receptors-and-opioid-receptor-painkillers

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen