Molecular mechanisms of opioid receptor signal transduction

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The analgesic and antidiarrhoeal uses of opium were known to the Sumerians and predynastic Egyptians. During 5000 years of medicinal use, opium has become associated with countries, cultures and prominent individuals, and through several modifications, remains an extensively used analgesic and addictive drug. Morphine, the principle active compound in opium, was first isolated by the German chemist Friedrich Sertuner in 1805. Replacing opium in therapy, its high potential for abuse was quickly discovered. Attempts to develop safer morphine analogues have spawned many compounds including heroin, a compound initially hailed as a safer and less addictive option. To date, however, no compound has proven free from a liability to be abused. Nevertheless, the search for safer morphine analogue led to the synthesis of the first antagonist, naloxone, and several other non-endogenous agonists and antagonists, effectively creating the first tools for opioid research. While pharmacological characterization is crucial, the search for safer analogues requires a thorough understanding of the mechanisms of cellular loss of responsiveness. The recent cloning of opioid receptors has created a new wave of research in the development of opioid tolerance, which may lead to a better understanding of this phenomenon.

Identification of opioid binding sites

Starting as early as 1954 with the experiments of Beckett and Casy, the concept of pharmacologically relevant opioid binding sites emerged and strengthened, with their discovery in mammalian brain. Research in the field has led to the identification of three distinct opioid receptors, μ, δ and κ, each possessing unique pharmacological and physiological properties. Although the existence of receptor subtypes has been proposed (for reviews see references 102 and 103), few data exist to support this. The first endogenous peptide ligands were discovered in the mid-1970s. Opioid ligands include enkephalins, dynorphins and endorphins, which are derived, in mammals, from three larger precursors: proenkephalin A, prodynorphin, and pro-opioi melanocortin, respectively. While several key experiments have resulted in extensive pharmacological characterization of opioid receptors, the presence of heterologous receptor populations and low expression in cells have always hampered further receptor studies. Recent cloning of the μ, δ and κ opioid receptors has allowed many questions to be answered by expressing receptor cDNAs in cell lines that lack endogenous receptors. While these studies have yielded invaluable data on receptor structure, the molecular nature of desensitization remains largely unresolved. A connection between cellular desensitization and drug tolerance has led countless scientists to puzzle over the molecular mechanisms controlling cellular adaptation. The long-term effect of agonists on the molecular mechanisms of signal transduction are important in understanding tolerance.

Biological effects of receptor activation

Agonist binding and subsequent receptor activation initiate a cascade of events that result in a varied array of biological effects. These include analgesia, miosis, bradycardia, general sedation, hypothermia, insensitivity and depression of flexor reflexes. The best studied role of opioids is in pain control, in which opioids are known to inhibit neurotransmitter release from dorsal root ganglion projections in the dorsal horn of the spinal cord. Opioids are also known to modulate endocrine processes and the immune system. This diversified repertoire prompts the obvious question: How can this system affect so many different processes? The cloning of opioid receptors confirmed that they belong to the G-protein-coupled receptor superfamily and therefore transduce their signals through interaction with guanine nucleotide-binding proteins. While knowledge of effector regulation by opioids precedes receptor cloning, the specificity involved in receptor regulation of these remains unclear. This review will describe the events leading to opioid-induced effector activation in the order they are presumed to occur.

Formation of receptor-ligand complexes

The biological effects exerted by opioid receptors begin with the formation of a specific receptor-
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ligand complex. Fuelled by the potential for discovering novel opioid ligands, many groups have searched for receptor domains involved in ligand selectivity. Comparison of the deduced primary structure of the opioid receptors shows a 65–70% homology between the three receptors, with highest homology among the transmembrane domains, intracellular loops and a small portion of the C-terminal tail near TM7. The second and third extracellular loops, and the N- and C-terminal tail, are highly divergent. To determine the regions of the receptors involved in ligand selectivity, initial studies relied on chimeric µ/κ or δ/κ receptors. Experiments swapping domains of the second and third extracellular loops found that these were important in ligand binding determination. Studies of chimeric receptors have shown that the third extracellular loop of δ opioid receptors,107 the first108 and third49 extracellular loops of the µ opioid receptor, and the second extracellular loop and top half of the fourth transmembrane domain in the κ opioid receptor25 61 108 115 are important in their respective agonist selective binding. These studies, however, were unable to reveal key residues in the receptor binding sites.

To determine the critical amino acids necessary for agonist and antagonist binding, receptor mutants with selective single amino acid substitutions were used. Aromatic transmembrane residues at positions 129 and 308,5 amino acids at positions 284, 296 and 297106 and 9530 of the δ receptor are all apparently necessary for binding of δ-selective ligands. Minami and colleagues42 identified a single residue, Lys108, that allows selective µ agonists to bind to δ receptors when mutated. Claude and colleagues20 found that the mutation of a single conserved serine in all three receptors (Ser196 at µ, Ser177 at δ and Ser187 at κ) confers full agonist properties on classical antagonists such as naloxone. While several residues have been found to be critical in the formation of a ligand–receptor complex, studies aimed at finding critical residues for receptor activation have been limited.

The association between opioid receptors and G-proteins was reported in the mid 1970s. Early studies showed that guanine nucleotides specifically decreased agonist binding in brain membranes5 and neuroblastoma X glioma (NG108-15) hybrid cells.6 It was noted that opioid receptors exist in two different affinity states depending on the presence or absence of guanine nucleotides. Detailed kinetic studies showed that the addition of GTP caused an increase in both agonist association and dissociation rates, but a greater increase in the dissociation rate.17 Evidence of naloxone-reversible, GTP-dependent inhibition of adenylyl cyclase by morphine and opioid-stimulated GTPase activity in NG108-15 cell membranes provided further evidence for G-protein coupling of opioid receptors.7 54 92 GTPase activity was initially associated solely with inhibition of adenylyl cyclase. However, regional differences between the distribution of GTPase activity and adenylyl cyclase inhibition were later reported.50 Opioid-induced GTPase activity is pronounced in hippocampus, cortex and striatum, while inhibition of adenylyl cyclase, appears to occur in striatum and frontal cortex. This suggests that agonist-induced GTPase activity is not only responsible for adenylyl cyclase regulation, but also for the regulation of other effector systems. This evidence later formed the basis for establishing that opioid receptors couple to multiple effector systems.

Opioid regulation of cyclic AMP

Cyclic AMP regulation by opioids was found in many cell lines and quickly became the trademark effect of opioid activity. Initially found in neuroblastoma X glioma cell lines by morphine treatment,92 adenylyl cyclase inhibition has been demonstrated in many cells expressing opioid receptors. This effect required both Na+ and GTP, like most other inhibitory receptor systems,7 and suggested the participation of inhibitory G-proteins. The role of G inhibitory trimeric G-proteins was proposed and confirmed in NG108-15 cells by the abrogation of coupling by pertussis toxin.40 Pertussis toxin (PT) can ADP-ribosylate and inactivate the inhibitory function of the α subunit, thus removing the inhibitory component of the pathway. Together with cholera toxin, which can ADP-ribosylate and thus inactivate the stimulatory function of the Go subunit, these compounds have proven invaluable in the identification of Gs subunit association with receptors. Adenylyl cyclase inhibition remains the effect most widely monitored to study acute and long-term effects of agonist treatment. To date, all cloned opioid receptors have been expressed in cell lines and found to inhibit this effect.44 56 114

Ion-channel regulation

Ion-channel regulation has further expanded the spectrum of opioid-induced biological effects. All three cloned opioid receptors couple to various Ca2+ channels (for a review see reference 78). These effects have been studied in a variety of cell types.41 72 Opioids can inhibit N- and P/Q-type Ca2+ channels in nucleus tractus solitarius84 and T-type Ca2+ channels in primary cultured dorsal root ganglion preparations.87 The cloning of several voltage-gated Ca2+ channels allowed several teams to express both opioid receptors and Ca2+ channels in Xenopus oocytes. From this, a specific Ca2+-channel α subunit involved in opioid receptor coupling has been identified.8 Piros and colleagues have shown that agonist-stimulated µ and δ receptors inhibit DHP-sensitive L-type Ca2+ channels.79 A recent study found that κ agonists modulate Ca2+ currents in isolated neuroendocrine nerve terminals.88 Opioid receptors are also known to regulate inwardly rectifying K+ channels. Experiments have shown agonist-mediated K+ channel regulation in Xenopus oocytes expressing both opioid receptors and K+ channels.38 Another study in lower vertebrates reveals that opioid receptors can activate K+ GIRK1 inwardly rectifying channels.24 Although K+–channel activation appears to be the norm, opioids can inhibit K+–channel activity in the F-ll cell line.22 28 Opioids also have measurable effects on Na+/H+ exchange.43

Secondary responses

In some cells, opioids can increase intracellular pools of Ca2+ by releasing intracellular stores. In these cells, 13
phospholipase C is activated upon agonist stimulation that induces the formation of the intracellular messengers IP3, and DAG. IP3 then promotes the release of Ca2+ from stores. However, information about opioid-induced phosphoinositol turnover is sparse and sometimes contradictory. Although one study has shown that κ opioid receptors stimulate phosphoinositol turnover in rat hippocampal slices, another has shown that κ receptors can inhibit GTPase-activated phospholipase C activity in guinea pig cerebellum.

Much work has gone into characterizing these secondary responses, yet little has been identified in terms of the signal transduction pathways generated from them. Opioids can provide mitogenic signals in a variety of cell lines. DADLE treatment of rat-1 fibroblasts expressing δ opioid receptor activates the p42 and p44 isoforms of MAP kinase — presumably a mitogenic signal leading to an increase in DNA synthesis.112 Opioid regulation of protein phosphorylation has also been observed. Although phosphorylation is common in brain membranes, several studies have shown opioid-specific inhibition of protein phosphorylation. Enkephalin treatment of brain membranes inhibited the phosphorylation of two membrane proteins of 47 kD and 10–20 kD (for review see reference 26). Nestler and co-workers70 have shown increased cAMP protein kinase activity in rat locus coeruleus in response to prolonged morphine administration.

Receptor-Effecter association

The existence of multiple effectors and biological responses mediated by opioids obviates the need for multiple signalling mechanisms. Opioid receptors expressed in cell lines have been shown to couple to a variety of trimeric G-proteins. Considering that receptor-activated effector selectivity is most probably mediated by these, a considerable amount of work has been carried out in to identify specific G-protein subunits associated with individual receptors. Presently, opioid receptors are thought to couple to Gs, Gi1, Gi2, Gi3, and presumably Gq G-protein subunits. The inhibitory actions of opioids on adenyl cyclase involve both Gi and Gq proteins. Gq, which has been implicated in κ-mediated analgesia,69 may be important in phospholipase C activation. Apparently Gi mediates Ca2+ release in cardiac myocytes by this mechanism.68 There are also several instances where hGTP-sensitive binding is not fully decreased by pertussis toxin, suggesting involvement of not only Gi2,38 but possibly also Gs proteins. Opioids exhibit stimulatory effects in certain instances (for a review, see reference 95). For example, there is evidence to suggest that opioid receptors may stimulate adenyl cyclase.15 In F-11 cells derived from dorsal root ganglia, opioids stimulate cAMP accumulation through G proteins sensitive to cholera toxin.22,28

A truly daunting task is establishing receptor-specific G-protein subunit association among the different receptors. Coimmunoprecipitation studies using transfected CHO cells have shown that the δ receptor associates with Gαi1, Gαi2, and Gαo in the native state, and dissociates from Gαi2 and Gαo and associates with Gαq2 upon agonist activation.57 Studies using CHO cells transfected with the κ opioid receptor have suggested that this receptor associates with Gαo, Gαq, and Gα2.80 Similar G-protein subunit distributions have been reported for μ and δ receptors.13,81 Although these experiments suggest that there are no receptor-specific associations with G-protein subunits, recent work shows that Gαq subunits insensitive to pertussis toxin act preferentially with μ receptors in the periaqueductal grey of mouse brain.31 Furthermore, while antibodies directed towards Gαq subunits blocked GTPase activity by all opioids, antibodies directed towards Gαi2 subunits blocked GTPase activity by DAMGO arid only slightly by δ agonists in periaqueductal grey.32

G subunit association studies have yielded conflicting results. Some studies have demonstrated specific receptor-G subunit association patterns that are similar for all opioid receptors, suggesting slight differences in receptor selectivity for Gi subunits among the different opioid receptors.81 Importantly, receptor-specific activation will lead to a specific biological response. Gs subunits may mediate such selectivity, as shown by the fact that only anti-Gαs antibodies can decrease Ca2+ current inhibition mediated by dynorphin A in dorsal root ganglia neurons.100 Although G protein subunits may be the basis of response specificity, activation signals can be further tailored by a vast and emergingly complicated pathway. Mammalian cells may express up to nine forms of adenylate cyclase, inhibition of this effector being the primary method to detect opioid activation. This multiplicity of adenyl cyclase isoforms may further target CAMP responses.39 Thirty forms of enzymes exhibiting cAMP phosphodiesterase activity have also been identified. Moreover, cell signalling has been enormously complicated by evidence of adenyl cyclase and other effector regulation by β γ subunits of G-proteins. Both β and γ subunits can inhibit adenyl cyclase, stimulate phospholipase C and activate inwardly rectifying K+ channels (reviewed in references 9 and 66). With more than five different β subunits and six γ subunits, a G-coupled protein receptor may selectively couple to any one of the permutations arising from the variety.69 As an example, somatostatin receptors inhibit voltage-gated Ca2+ channels via α,β,γ.31 The situation may therefore be far more complex than initially imagined.

Ligand-induced conformational changes

Ligand-induced conformational changes may also play a role in receptor-induced biological responses. Evidence of this may lie in the puzzling findings that agonists with similar affinities can have different potencies.

An interesting study found that the delta-selective antagonist ICI-174,864 could potentiate forskolin-induced cAMP accumulation in HEK-293 cells transfected with the delta receptor.18 These cells otherwise exhibited normal inhibition of adenyl cyclase by (P-Pen2,P-Pen5)enkephalin. Morphine is unable to internalize the μ receptor in transfected cells while retaining the ability to inhibit adenyl cyclase.48 One study has shown that while the specific agonist U69,593 was able to cause receptor down-regulation of κ opioid receptor expressed in CHO

cells, the non-specific opioid agonist levorphanol did not.\(^4\) These results suggest that agonist-induced conformational changes are important in receptor regulation, which may be related to signal termination. It is not unreasonable to suggest that different agonists could have different effects on G-protein coupling, leading to different biological responses. Further structural studies may yield answers to this question.

**Spatial aspects of response modulation**

Although three opioid receptors, several possible receptor subtypes, \(G_\alpha, G_\beta, G_\gamma\) and ligand-induced modulation suggest that an enormous number of factors is involved in the activation of a specific response, there may be a simpler explanation. Most studies are conducted in transfected cells containing several, and not a physiologically “select” group of G proteins, thus disregarding G-protein availability and spatial considerations. Few processes occur in isolation or irrespective of the spatial location of factors involved in signal transduction and termination. Specificity could simply be a function of protein availability. Mammalian cells have the option of expressing any one of nine isoforms of adenylyl cyclase and 30 forms of enzymes with cAMP phosphodiesterase activity, making specificity an act of presence. Spatial relevance within membrane limits has been demonstrated in an elegant study by Wilding and colleagues.\(^{106}\) By simultaneously recording the channel activity within a patch and in the whole cell, they showed that bath applications of DAMGO on the cell, and not inside the patch, affected the whole-cell \(Ca^{2+}\) currents and did not affect \(Ca^{2+}\) channel activity within the patch. The inhibition of \(Ca^{2+}\) channels by DAMGO treatment therefore occurs only between closely associated opioid receptors and \(Ca^{2+}\) channels. Although a direct interaction between the G protein and the adjacent channel is probably responsible for such spatial regulation, direct interaction between receptor and channel is not an unreasonable suggestion.

**Agonist-induced opioid desensitization**

Once an intracellular message has been sent, opioid receptors will cease to translate extracellular messages. This is a crucial event in the regulation of extracellular signals via G-protein-coupled receptors. Cellular responses to stimulation are usually rapidly decreased or terminated to return to homeostasis. These concepts are extremely important as the principles of cellular adaptation are thought to govern drug tolerance. While we have extensive knowledge of how G-protein-coupled receptors become desensitized to their environment, surprisingly little is understood about the mechanisms of agonist-induced opioid desensitization.

Desensitization is defined as a loss of function under prolonged exposure to an agonist. This suggests that any of the factors involved in effecting a biological response (effectors, receptors and messengers) may be subject to regulation. Volumes of information exist regarding cellular desensitization to opioids. Most of this work has been performed by measuring the attenuation of secondary responses upon agonist stimulation. By far the most studied opioid-induced cellular attenuation is that of adenylyl cyclase activity. Several early studies on desensitization of adenylyl cyclase inhibition were performed on cells exposed to prolonged morphine treatment. An initial loss of intracellular cAMP concentrations followed by a gradual increase in cAMP levels was observed in these experiments. These effects were receptor mediated because the addition of antagonists could restore cAMP levels. Interestingly, if morphine was removed or an antagonist added after 12 h, cAMP concentrations increased to higher than original levels. This was proposed to be the cellular basis for opiate withdrawal (a transient cAMP overshoot). Several other studies have shown that prolonged treatment with agonist reduces the receptor-ligand affinity and uncouples the system from adenylyl cyclase. In NG108-15 cells, 1 h of DADLE treatment was enough to desensitize GTPase activity by 65% but adenylyl cyclase activity was decreased only 20%, suggesting that GTPase activity is involved in other effects in addition to adenylyl cyclase inhibition.\(^{106}\) It is interesting that while studies have found strong evidence of \(\delta\) and \(\mu\) receptor uncoupling from adenylyl cyclase, there are conflicting data on the \(\kappa\) opioid receptor. Several reports indicate that while prolonged agonist treatment alters \(\kappa\)-specific ligand affinity, \(\kappa\) receptors do not undergo desensitization as measured by inhibition of adenylyl cyclase. These studies have been performed in the R1.1 mouse thymoma cell line\(^6\) and in transfected CHO cells.\(^2\) Other studies measuring the desensitization of electrophysiological effects have shown the contrary. Prolonged treatment of \(\kappa\) agonist U50, 488 on AtT-20 cells transfected with \(\kappa\) receptors resulted in the uncoupling of the receptor from an inwardly rectifying \(K^+\) ion channel.\(^39\)

**MECHANISMS OF DESENSITIZATION**

Although desensitization has been observed extensively, little is understood about the mechanisms by which it occurs. A system may become desensitized by global or local regulation or, more likely, by both. There are several important questions that must be addressed. Do opioids lose their ability to inhibit adenylyl cyclase because of effector inactivation? Or are cellular GTP levels decreased? Do the receptors themselves desensitize? If they do, does this involve structural modifications or simply regulation of receptor quantities? While most of the signalling attenuation may be directly related to the desensitization of the opioid receptor, this could not explain the ability of \(\mu\) and \(\delta\) agonists heterologously to desensitize cells. Prolonged \(\mu\) agonist treatment of transfected cells not only rendered the system unable to activate \(K^+\) channels upon further DAMGO addition, but also upon addition of MK 678, a somatostatin agonist. This suggests a global desensitization of the cell upon \(\mu\) agonist treatment. Adenylyl cyclase may therefore become uncoupled not because of receptor desensitization but because of a global regulation of key factors.

While it seems trivial to discuss receptor desensitization when tolerance exists at the level of the cell and therefore the organism, drugs are being designed that reversibly bind to receptors without triggering cellular adaptation. If receptor desensitization trig-
gers a generalized cellular adaptation, it will be important to separate and understand the individual events leading to the cellular loss of response to opioids. The apparent inability of κ opioid receptors to become desensitized after prolonged agonist treatment may be related to the relative low abuse liability associated with κ agonists. As a thorough description of desensitization is beyond the scope of this article, we shall describe the mechanisms of receptor regulation that may be crucial to cellular adaptation. Opioid receptor regulation is fundamental for signalling control and involves internalization, down-regulation and possible receptor modifications.

MECHANISMS OF OPIOID RECEPTOR REGULATION

Agonist-induced receptor internalization and down-regulation are crucial parts of receptor regulation and may form the basis of cellular adaptation to acute and long-term agonist treatments.

Agonist-induced rapid internalization

Demonstration of agonist-induced rapid internalization of other G-protein-coupled receptors suggested a similar mechanism of receptor regulation for opioid receptors. Opioid receptors expressed in heterologous cells undergo rapid internalization upon agonist treatment; approximately 50% of the receptors are internalized within 6 min, as measured by flow cytometry. The rapid internalization of μ receptors in response to high-affinity agonists such as etorphine has also been observed in neurons in vivo. The agonist-mediated internalization is homologous because δ opioid receptors internalize in response to δ receptor selective ligands and not to μ or κ receptor selective ligands and vice versa. It is interesting that κ opioid receptors do not exhibit rapid internalization in response to treatment with high-affinity agonists such as etorphine.

Little is known about the molecular mechanisms of this rapid internalization or the domains of the receptor involved in this phenomenon. We have recently found that δ opioid receptors exist as a dimer and undergo agonist-mediated monomerization. Receptor monomerization precedes internalization, suggesting that agonist-induced monomerization may be a prerequisite for receptor internalization. The C-terminal tail is necessary for dimerization and internalization, as the deletion of a portion of the C-tail results in a loss of both dimerization and internalization. Mutation of any one of the serine/threonine residues in the C-tail results in significant loss of internalization, suggesting that these residues play an important role in modulating receptor conformation and thus affecting internalization. A recent study has found that a nine-residue truncation of a Ser/Thr-rich domain unique to the C-term of μ receptors results in a constitutively internalizing and recycling mutant. Taken together, these studies underscore the importance of the C-tail in opioid receptor internalization.

Receptor down-regulation

Receptor down-regulation is an important method by which cells become desensitized to prolonged agonist treatment. It is thought to cause a development of drug tolerance and may result from alterations of the rates of degradation and synthesis. Down-regulation is characterized by a generalized loss of both cell-surface and intracellular receptors. Prolonged antagonist treatment is known to have opposite effects mediating an upregulation of opioid receptors. Down-regulation of opioid receptors has been demonstrated in whole animals, neuronal cell lines and transfected cells. Although long-term regulation of opioid receptors is thought to proceed from modulation of mRNA synthesis, this is still largely disputed. One study found that morphine treatment caused a significant decrease in μ-opioid receptor mRNA in basal hypothalamus, while leaving these mRNA levels unchanged in the preoptic area and thalamus. Another study found that a marked down-regulation of δ opioid receptor binding sites after a 24-h treatment was not accompanied by a decrease in receptor mRNA levels in the thalamus. A third study found that prolonged etorphine treatment down-regulates both the δ opioid receptor binding sites and mRNA levels in NG108-15 cells.

While the molecular basis of down-regulation is not well understood, a recent study has implicated a receptor/G-protein complex as a necessity for down-regulation. This study found that μ opioid receptors required a high-affinity G/complex formation to internalize and down-regulate, as assessed by the ability of pertussis toxin to block internalization. Surprisingly, this study also found that δ opioid receptor down-regulation was not affected by pertussis toxin treatment.

Phosphorylation may also be important in receptor down-regulation; this is shown by mutations of Thr353 of the carboxy tail of the δ opioid receptor that markedly affect down-regulation. Receptor inactivation by phosphorylation has been reported in several G-protein-coupled receptors, but remains unclear in the opioid system. Several studies acknowledge phosphorylation of opioid receptors, yet the role of this modification in receptor regulation or activation is not clear. The regulation of β-adrenergic receptor and other G-protein-coupled receptor levels by phosphorylation suggests that this is likely for opioid receptors. Phosphorylation by G-protein-coupled receptor kinases (GRKs) or cAMP responsive kinases or both could play a significant part in receptor down-regulation.

Physiological relevance of studies of the opioid system

Now that receptor cloning has made transfected cell lines a convenient method by which to study the opioid system, the impending physiological relevance must be carefully assessed. One must note that high expression systems are non-physiological and have increased populations of spare receptors, a phenomenon that may alter pharmacological studies. Several studies have shown the important effect of spare receptor populations on agonist affinity. One study has shown that the irreversible blocking of 95% of opioid receptor binding sites by β-chlormethylamine (β-CNA) did not alter the inhibition of adenyl cyclase as mediated by opioid agonists. Furthermore, it has been shown that the receptors exist in...
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different affinity states, not only as a function of GTP and Na\(^+\), but also as a function of receptor occupancy.\(^{55}\) Desensitization studies measuring inhibition of adenyl cyclase after prolonged agonist treatment have often neglected the presence of spare receptors. The presence of many unoccupied receptors, each with the ability to inhibit adenyl cyclase, may yield ambiguous clues as to the specific desensitization of the opioid receptor. More factors to complicate desensitization studies lie in the broad subject of opioid peptide regulation, which is beyond the scope of this review.

In summary, the complexity of the opioid system was widely understood even before the receptors were cloned. The emerging complex functions of trimeric G-proteins and other effector systems have provided many challenges for all G-protein coupled receptors. Although the existence of opioid receptors has been recognized for almost 30 years, exploration of receptor regulation is still at an early stage. Future work should unravel unanswered questions and possibly lead to an explanation of the molecular mechanisms of opioid tolerance.

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