Published in final edited form as:

Ann Neurol. 2010 May ; 67(5): 559–569. doi:10.1002/ana.22002.

How to Design an Opioid Drug That Causes Reduced Tolerance and Dependence

Amy Chang Berger, BS and Jennifer L. Whistler, PhD

Department of Neurology, University of California, San Francisco, Ernest Gallo Clinic and Research Center, Emeryville, CA

Abstract

Mu opioid receptor (MOR) agonists such as morphine are extremely effective treatments for acute pain. In the setting of chronic pain, however, their long-term utility is limited by the development of tolerance and physical dependence. Drug companies have tried to overcome these problems by simply “dialing up” signal transduction at the receptor, designing more potent and efficacious agonists and more long-lasting formulations. Neither of these strategies has proven to be successful, however, because the net amount of signal transduction, particularly over extended periods of drug use, is a product of much more than the pharmacokinetic properties of potency, efficacy, half-life, and bioavailability, the mainstays of traditional pharmaceutical screening. Both the quantity and quality of signal transduction are influenced by many regulated processes, including receptor desensitization, trafficking, and oligomerization. Importantly, the efficiency with which an agonist first stimulates signal transduction is not necessarily related to the efficiency with which it stimulates these other processes. Here we describe recent findings that suggest MOR agonists with diminished propensity to cause tolerance and dependence can be identified by screening drugs for the ability to induce MOR desensitization, endocytosis, and recycling. We also discuss preliminary evidence that heteromers of the delta opioid receptor and the MOR are pronociceptive, and that drugs that spare such heteromers may also induce reduced tolerance.

Opioids have been used to treat pain for well over 5,000 years.¹ Before the 19th century, the drug of choice was opium, the resin released from the seed pods of the opium poppy. The potency of opium varied greatly from batch to batch, making it impossible to standardize dosage and greatly increasing the risk of overdose. The purification of morphine from opium in 1803 finally solved this problem and greatly improved the safety of using opioids; however, once drug doses were easily quantifiable, the problem of tolerance, defined as the need for higher doses of drug to achieve the same effect, became increasingly apparent. Tolerance was frequently accompanied by physical dependence, the need for continued drug use to prevent somatic and affective withdrawal symptoms, and in some cases by addiction.

Scientists have assumed, or at least hoped, that the biological mechanisms mediating opioid analgesia are distinct from, and can be divorced from, those mediating tolerance, dependence, and addiction, and from the time of morphine's discovery, they have tried to develop opioids with a reduced propensity to cause these negative sequelae of prolonged opioid use. Two hundred years later, however, morphine is still a mainstay of modern pain management, and

the satisfactory treatment of chronic pain is still, in many cases, frustrated by tolerance as well as fear of dependence and addiction.

Many attempts to develop a better opioid have been based on flawed or incomplete hypotheses of tolerance development. As a case in point, both heroin and sustained release oxycodone (trade name OxyContin), today 2 of the most highly sought after opioid drugs of abuse, were initially marketed as promoting less tolerance and habit formation than morphine. Fortunately, our understanding of the molecular mechanisms underlying opioid analgesia as well as tolerance and dependence has improved greatly, paving the way for a new generation of more rationally designed and screened opioid analgesics.

If the goal is truly to develop a better opioid and not “Band-Aid” drugs to treat the undesired effects of opioids, then one must start at the opioid receptor. The biological actions, both desired and undesired, of morphine and other narcotic analgesics are mediated primarily by the mu opioid receptor (MOR), as they are abolished by MOR-specific antagonists and in MOR knockout (KO) mice.² In our laboratory, we study the effect of MOR trafficking and hetero-oligomerization on opioid analgesia, tolerance, and dependence.

Regulation of Signal Transduction by MOR Trafficking

The MOR is a member of the $G_{i/o}$ -coupled family of G protein-coupled receptors (GPCRs). When the MOR is activated, for example by endogenous enkephalins and β -endorphin, it signals by catalyzing nucleotide exchange on G_i and G_o , leading to inhibition of adenylyl cyclase, neuronal hyperpolarization via activation of K^+ channels, and inhibition of neurotransmitter release via inhibition of Ca^{2+} channels. Following activation, receptors are rapidly phosphorylated by GPCR kinases (GRKs) and subsequently bound by arrestin. These 2 events uncouple the MOR from G protein, resulting in receptor desensitization. Arrestin additionally recruits clathrin and other components of the endocytic machinery, and endocytosis further attenuates receptor signaling by removing ligand-receptor complexes from the cell surface. Finally, internalized MORs are resensitized by rapid recycling to the plasma membrane (for review, see Ferguson et al³).

In this cycle, endocytosis acts as an off-on-off-on timer that precisely titrates MOR signal transduction. First, when the receptor is actively signaling, receptor desensitization and endocytosis function as an off switch that rapidly silences receptor activity. However, once the receptor has been desensitized, endocytosis and recycling act as an on switch that is necessary for the recovery of agonist responsiveness.

The off-on function normally provided by endocytosis may be compromised in response to morphine and other commonly used opioids, including heroin and oxycodone. Whereas endogenous opioids promote robust MOR internalization, morphine fails to induce substantial endocytosis in vitro, even after prolonged periods at saturating concentrations.^{4,5} Morphine also fails to induce MOR endocytosis in multiple brain regions in vivo, including those involved in pain transmission and morphine reward, such as the dorsal horn of the spinal cord, the periaqueductal gray matter (PAG), and the ventral tegmental area (VTA).^{6–9} Importantly, in preparations where morphine does promote some endocytosis, the degree of internalization is substantially less than that achieved with endogenous peptide ligands and certain synthetic agonists, such as methadone.^{10,11}

Defects in agonist-induced internalization could impact MOR signaling and contribute to the development of morphine tolerance and dependence in multiple ways (Fig 1). The direction of the effect would depend on whether or not the receptor has already been desensitized. If the receptor has been desensitized by phosphorylation and/or arrestin binding, then the failure to resensitize, that is, the loss of the on function provided by endocytosis, would result in the

persistent loss of signaling-competent receptors and could manifest as acute or chronic tolerance. In this case, agonists that do not induce desensitization, agonists that induce both desensitization and resensitization via receptor trafficking, or highly efficacious agonists that function at low receptor occupancy and high receptor reserve would be expected to drive less tolerance. On the other hand, if the receptor has not been desensitized, then the failure to internalize, that is, the loss of the off function provided by endocytosis, would result in persistent receptor activation, and such abnormally prolonged signaling could, in turn, trigger homeostatic cellular and systemic adaptations that manifest not only as tolerance but also as physical dependence. In this case, agonists that induce desensitization, endocytosis, and recycling would be expected to drive both less tolerance and less dependence. Thus, regardless of whether opioid tolerance is due to impaired on function (persistent desensitization due to poor endocytosis and resensitization) or impaired off function (persistent activation due to poor desensitization and endocytosis), agonists that promote MOR trafficking similar to endogenous ligands would be expected to produce less tolerance.

Persistent MOR Desensitization and Tolerance

The hypothesis that MOR desensitization is the cellular basis of behavioral tolerance has been studied extensively by our laboratory and others. In general, morphine-occupied receptors elude GRK- and arrestin-mediated desensitization in heterologous expression systems and brain slices *in vitro*.^{12,13} However, the endogenous complement of GRKs and arrestins may vary across cell types and brain regions.^{14,15} *In vivo* studies using chronic morphine have detected desensitization in some cases^{16–19} but not others.^{20–22} Similarly, attempts to reduce tolerance by genetically ablating either GRKs or arrestins have produced mixed results. GRK3 KO mice have reduced tolerance to fentanyl but not to morphine, consistent with evidence that morphine does not stimulate robust GRK phosphorylation,²³ whereas β -arrestin 2 KO mice have reduced tolerance to morphine and heightened sensitivity to the analgesic effects of a single dose of morphine and heroin but not fentanyl or methadone.^{24–26} These seemingly paradoxical results can be reconciled by the fact that fentanyl and methadone are stronger MOR internalizers than morphine and can recruit additional desensitizing mechanisms, such as β -arrestin 1, to the receptor in the absence of β -arrestin 2.^{10,26,27} Importantly, neither GRK nor arrestin deletion reduces the development of opioid dependence and withdrawal.^{23,25}

It is safe to say that compared to endogenous opioid peptides, morphine is a poor inducer of canonical desensitization mediated by GRKs and arrestins. However, under certain conditions, other kinases, most notably protein kinase C (PKC), may participate in MOR desensitization.^{28,29} PKC does not appear to play a role in persistent desensitization caused by acute morphine, as PKC inhibitors do not enhance acute morphine analgesia.^{30,31} Rather, PKC-mediated desensitization appears to be a secondary mechanism to reduce persistent MOR activation when GRK phosphorylation, arrestin binding, and/or endocytosis are inhibited or fail, as in the case of morphine. Furthermore, although analgesic doses of morphine do not activate PKC in naive animals, chronic morphine treatment has been shown to increase coupling of the MOR to pronociceptive PKC-dependent signaling pathways.^{32,33} PKC-dependent tolerance to DAMGO, a synthetic analogue of endogenous enkephalin, is only observed when endocytosis is inhibited,³⁴ and similarly, deletion of arrestin is required to unmask PKC-dependent tolerance to morphine in the spinal cord.¹⁵ This may be explained by the loss of arrestin-dependent translocation of the MOR away from lipid rafts where it is normally retained, as it has been shown that localization within rafts is necessary for PKC activation by some GPCRs, including the NK1 receptor.^{35,36} Whereas GRK-phosphorylated receptors are rapidly resensitized by endocytosis and recycling, PKC activation inhibits endocytosis, and PKC-phosphorylated receptors appear to require the activity of phosphatases to restore signaling competency.^{37–39} Thus, PKC phosphorylation may be a response to unusually prolonged MOR activation that leads in turn to unusually prolonged desensitization.

Overall, a complex picture is emerging in which morphine does induce limited MOR desensitization in certain brain regions in vivo, including the brainstem, an important site for pain transmission.^{25,40} Although morphine recruits desensitizing mechanisms poorly compared to endogenous opioids and drugs like methadone, the functional impact is amplified, perhaps by a deficit in receptor endocytosis and resensitization. Persistent desensitization thus contributes to acutely diminished sensitivity to morphine, occurring within minutes of an animal's first exposure to drug, and to tolerance, particularly in situations where high doses of drug given over a short period of time induce the development of tolerance over hours to days, such as in many animals models. However, in patients receiving opioid analgesics, tolerance typically emerges on the time scale of weeks. Such clinical tolerance is most likely due not only to true insensitivity to medication caused by desensitization but also to increased pain caused by homeostatic changes in signal transduction, as discussed below.

Persistent MOR Activation and Dependence

By their very nature, opioid dependence and withdrawal cannot be explained by receptor desensitization. The fact that withdrawal from opioid agonists promotes a physiological response, especially in profoundly tolerant individuals, is proof that at least some opioid receptors are still actively signaling. In other words, if all receptors were desensitized, then removing drug from them would produce no effect. However, the administration of a MOR antagonist to dependent subjects, such as heroin addicts, unmasks active withdrawal symptoms, such as pain, agitation, and diarrhea, that are the polar opposite of the acute effects of opioids. In dependent subjects, therefore, the body has adapted to persistent high opioid tone by making homeostatic adjustments in antiopioid systems. Such compensatory changes have been observed at both the cellular level, for example, cyclic adenosine monophosphate (cAMP) superactivation, and the circuit level, for example, increased glutamate transmission in excitatory nociceptive pathways (for review, see King et al⁴¹ and Williams et al⁴²).

The homeostatic adaptation that is perhaps best studied is cAMP superactivation. In response to acute treatment with MOR agonists, cAMP production by adenylyl cyclase is inhibited, protein kinase A (PKA) activation is reduced, and levels of phosphorylated cAMP response element-binding protein (CREB) fall. In contrast, chronic morphine exposure leads to an increase in the expression of certain isoforms of adenylyl cyclase, PKA, and CREB.⁴³ In addition, functional upregulation of the cAMP pathway has been demonstrated in multiple brain regions, including the locus coeruleus (LC),⁴⁴ the PAG,⁴⁵ the nucleus accumbens (NAc),⁴⁶ and the VTA.^{44–47} Intriguingly, injection of an opioid antagonist into these sites in morphine-dependent animals elicits many of the somatic (LC and PAG) and motivational (NAc) symptoms of withdrawal.^{48–50}

cAMP superactivation may in fact be the cellular basis of opioid withdrawal. Administration of CREB antisense oligonucleotides or PKA inhibitors directly into the LC or PAG attenuates the somatic signs of withdrawal in morphine-dependent animals. Conversely, in morphine-naïve animals, administration of PKA activators precipitates a withdrawal-like syndrome.^{44, 51} cAMP superactivation also leads to an increase in the excitability of MOR-expressing neurons and an increase in neurotransmitter release from these cells. For example, chronic morphine and withdrawal cause a cAMP-dependent increase in γ -aminobutyric acid synaptic transmission in interneurons in the NAc,⁴⁶ dopaminergic neurons in the VTA,⁴⁷ and pain inhibitory neurons in the PAG^{22,45} and nucleus raphe magnus (NRM).^{22,45,52} Each of these effects opposes the acute action of morphine, and on removal of morphine, they may contribute to rebound excitation that exceeds premorphine levels.

When opioids are used for a prolonged period of time, as in the treatment of chronic pain, some amount of compensation and hence dependence may be inevitable. However, just as with

tolerance, not all opioids are created equal when it comes to dependence. For example, methadone produces a much milder withdrawal syndrome than morphine when administered chronically to otherwise opioid-naïve animals.⁴⁰ In general, MOR agonists that stimulate robust receptor internalization induce less tolerance and dependence in vivo when administered at equianalgesic doses.^{53–55}

The Mutant Recycling MOR

As mentioned above, studies comparing different MOR agonists have found that those that induce robust receptor endocytosis and recycling, and thus a response more like that induced by endogenous ligands, tend to produce reduced tolerance and dependence in vivo. Based on these pharmacological studies alone, however, it is impossible to conclude that endocytosis per se is protective, because different agonists differ in other properties in addition to endocytosis, including specificity, potency, efficacy, and half-life.

To isolate the effect of endocytosis on morphine analgesia, tolerance, and dependence, we generated a mutant recycling MOR (RMOR) that desensitizes, internalizes, and recycles in response to morphine.⁵⁶ Importantly, the affinity of the RMOR for morphine and other MOR ligands and the efficiency of its coupling to G protein are equivalent to the wild-type (WT) receptor. The trafficking of the RMOR in response to peptide ligands and methadone are also unchanged. Initial in vitro experiments demonstrated that cultured epithelial cells expressing the mutant receptor develop reduced signs of cellular tolerance and withdrawal, including cAMP superactivation and CREB-induced gene expression. To determine whether this would translate into reduced systemic tolerance and withdrawal, we generated a knock-in mouse line that expresses the mutant RMOR in place of the WT receptor.⁴⁰

RMOR mice have normal baseline responses to painful stimuli, suggesting that their basal opioid tone is unchanged. In addition, they show no differences in MOR expression or distribution in the central nervous system. However, the same dose of morphine produces a greater analgesic response in RMOR mice than in WT mice, suggesting that endocytosis does reduce rapid receptor desensitization, presumably by facilitating resensitization through receptor recycling. In fact, biochemical analysis of neuronal membranes revealed significant receptor/G protein uncoupling in the brainstem of WT but not RMOR mice after a single morphine treatment. Together, these data suggest that morphine does induce some MOR desensitization in WT mice that is alleviated by promoting receptor endocytosis and recycling.

RMOR mice also develop markedly reduced tolerance and physical dependence following repeated morphine administration, suggesting that endocytosis reduces compensatory upregulation of antiopioid systems. Indeed, we have observed, at the cellular level, reduced cAMP superactivation in the striatum⁵⁷; at the synaptic level, reduced inhibitory neurotransmission onto midbrain dopaminergic neurons⁵⁸; and at the systemic level, reduced expression of glucocorticoid hormone receptors.⁵⁷

If improved agonist-induced endocytosis is, in fact, responsible for the phenotype of RMOR mice, then one would expect drugs that stimulate internalization in WT mice to produce equal responses in both genotypes. In fact, methadone produces equivalent analgesia and mild dependence in WT and RMOR mice. Thus, the RMOR mice provide compelling evidence that MOR endocytosis protects against the development of opioid tolerance and dependence. Experiments are currently underway to determine whether it likewise protects against addiction.

Morphine Cocktails That Promote MOR Endocytosis and Recycling

A genetic mutation that facilitates MOR recycling is out of reach for most clinicians and patients seeking relief from chronic pain (although, intriguingly, just such a mutation has been described in human subjects and causes reduced cAMP superactivation in response to chronic morphine *in vitro*⁵⁹). Opioid drugs that induce MOR endocytosis to a greater degree than morphine are available; however, all of them have properties that make them undesirable for long-term use (see Appendix).

To convert morphine into a safer and more effective drug, we sought to take advantage of another property of the MOR that influences its signal transduction and trafficking. Like other GPCRs, the MOR is thought to exist as a dimer or higher order oligomer. The MOR can oligomerize with itself⁶⁰ or with other GPCRs, including the cannabinoid CB1 receptor,⁶¹ the substance P receptor,⁶² the serotonin 5HT2A receptor,⁶³ and other opioid receptors.⁶⁰ In several cases, it has been demonstrated that co-expression of another receptor with the MOR alters the trafficking of the MOR. For example, when the WT MOR forms a heteromer with a mutant MOR that internalizes in response to morphine, the WT receptor is dragged along and also internalizes with morphine.⁶ Similarly, when the MOR is coexpressed with the 5HT2A receptor, morphine promotes MOR internalization in the presence of serotonin.⁶³

The precise mechanism of this dragging is unclear; however, there are at least 2 possibilities. First, heterooligomerization may directly alter the conformation of the MOR, making it a better substrate for desensitization by kinases and arrestins. Alternatively, the binding partner in the heteromer may simply recruit a high concentration of kinases and arrestins to the vicinity of the MOR, and this may be sufficient to tip trafficking in the right direction. The latter possibility seems likely to play at least a partial role, as overexpression of either GRK or arrestin is sufficient to promote morphine-induced endocytosis of MOR homomers.^{12,13} Either way, the dragging phenomenon suggests that in a MOR homomer, if one protomer were bound to morphine and 1 protomer were bound to methadone or another ligand that promotes endocytosis, then the entire oligomer might undergo endocytosis.

We tested this hypothesis by combining morphine with either methadone or DAMGO, a synthetic enkephalin analogue. Both morphine cocktails induced MOR endocytosis *in vitro* and *in vivo*, even when the concentration of methadone or DAMGO used was so low that it produced no endocytosis on its own.^{6,7} Even more remarkably, rats treated chronically with a combination of morphine and a low, subanalgesic dose of methadone developed greatly diminished tolerance and dependence compared with rats treated with the same concentration of morphine alone (Fig 2).^{7,64} Similarly, an independent group found that coadministration of morphine with subanalgesic doses of either fentanyl or DAMGO produced greater analgesia than morphine alone.⁶⁵ Like DAMGO, endogenous opioid peptides would be expected to facilitate the internalization of morphine-occupied MORs, and in fact, in the setting of tissue injury, endorphins and enkephalins released by inflammatory cells have been shown to promote MOR trafficking in peripheral sensory neurons and to inhibit tolerance to locally applied morphine.⁶⁶ These results provide confirmation that MOR endocytosis delays the onset of tolerance and dependence to opioids. They also provide proof-of-concept that existing, widely available, and inexpensive opioid drugs, with known drug interaction and side effect profiles, can be combined in ways that preserve their most desirable pharmacologic and endocytic properties. Clinical trials are currently in preparation to determine whether morphine cocktails that have been shown to promote endocytosis in preclinical models cause reduced tolerance compared to morphine alone, when used to treat postoperative pain.

Opioid Receptor Heteromers and Tolerance

A MOR homomer in which 1 protomer is occupied by morphine and the other by methadone has distinct signaling and trafficking properties from a homomer that is occupied by morphine alone. It is, in effect, a functional MOR heteromer. Opioid receptor heteromers in the classical sense may also represent attractive targets for the next generation of opioid drugs. In vitro, heterooligomerization has been shown to alter ligand binding properties, trafficking, and even the identity of signaling partners and second messenger systems.^{6,67–70} However, the in vivo study of opioid receptor heteromers is still in its infancy, and even the existence of endogenous heteromers is controversial, because highly selective ligands are not available.

We have identified a heteromer-selective agonist, 6'-guanidinonaltrindole (6'-GNTI), that potently activates heteromers composed of the delta opioid receptor (DOR) and kappa opioid receptor (KOR) but not homomers of either receptor alone. 6'-GNTI is a potent analgesic when administered intrathecally; however, intraventricular administration produces no effect, suggesting that the expression of antinociceptive DOR/KOR heteromers is restricted to the spinal cord.⁷¹ 6'-GNTI thus demonstrates the presence and physiological relevance of endogenous opioid receptor heteromers and provides proof-of-principle that safer, more effective opioid drugs may be developed by targeting heteromers whose expression is limited to specific tissues and circuits.

For the purpose of developing opioids that induce reduced tolerance, we are particularly interested in heteromers containing the DOR. Chronic morphine stimulates the translocation of quiescent, intracellular DORs to the plasma membrane in multiple sites that are important for pain transmission, including the dorsal root ganglia, spinal cord,^{72,73} PAG,⁷⁴ and NRM.⁷⁵ Multiple lines of evidence indicate that this functional upregulation of the DOR plays a central role in morphine tolerance. Mice deficient in either the DOR itself,^{76–79} its endogenous ligand,^{78,80} or preprotachykinin,⁷³ which is required for the proper membrane expression of the DOR, all develop reduced tolerance to morphine. Furthermore, combined treatment with morphine and a DOR antagonist induces less tolerance than morphine alone, whereas treatment with morphine and a DOR agonist induces greater tolerance.^{81,82}

To date, however, the DOR has been largely dismissed as a target for the treatment of pain, because DOR ligands produce equivocal results in vivo. For example, DOR-mediated analgesia is generally much weaker than that mediated by MORs,^{83,84} and in the spinal cord, both DOR agonists and antagonists have been found to enhance morphine analgesia.^{69,85–87} We believe this is due to the presence of multiple DOR subtypes with opposing actions.

At least 2 pharmacologically distinct DOR subtypes have been described in vivo, DOR1 and DOR2. In the brain, the DOR1-selective agonist, DPDPE, is a less potent analgesic than the DOR2-selective agonist, deltorphin.^{88,89} Although DPDPE produces weak analgesia on its own, the DOR1-selective antagonist, DALCE, also produces analgesia and is 2 orders of magnitude more potent, suggesting that a substantial portion of supraspinal DOR1 receptors are pronociceptive.⁹⁰ We recently discovered that DOR1 and DOR2 also have opposing effects on ethanol consumption, suggesting that bidirectional control of behavior may be a common feature of DOR-containing circuits. We found that the DOR1-selective agonist TAN-67 and the DOR2-selective antagonist naltriben both decrease drinking, whereas the non-selective DOR antagonist naltrindole has no effect, as would be expected if DOR1 and DOR2 antagonize each other.⁹¹

The molecular basis of DOR subtypes is not known; however, we believe that heteromers are a likely candidate. The distinct pharmacological profiles of the DOR1 and DOR2 cannot be recapitulated in heterologous cells expressing only the DOR. However, in cells expressing both the MOR and DOR, the affinity and potency of DOR1 ligands are selectively increased,

whereas those of DOR2 ligands are decreased, suggesting that the DOR1 subtype may be a DOR/MOR heteromer.⁹¹ Putative DOR/MOR heteromers have been detected in mouse spinal cord membranes,⁶⁹ and their existence is further supported by data from opioid receptor KO mice.^{76,92} For example, we found that the effect of TAN-67 on ethanol consumption was lost in both DOR and MOR KO mice, whereas the effect of naltriben was lost in DOR KOs but preserved in MOR KOs and KOR KOs, once again suggesting that DOR1 may be a DOR/MOR heteromer, whereas DOR2 may be a DOR homomer.⁹¹

We are currently conducting experiments to determine whether DOR1s or DOR2s are preferentially up-regulated following chronic morphine treatment. Paradoxically, morphine induces at least some antinociceptive DOR2s that are activated by deltorphin and that would be expected to enhance rather than inhibit morphine analgesia.^{72,75} However, any analgesia provided by DOR2s diminishes after more prolonged morphine treatment,^{93,94} and chronic morphine has also been shown to induce the emergence of pronociceptive opioid receptors that couple to excitatory G_s rather than inhibitory G_i and that are potently inhibited by both DOR- and MOR-specific antagonists, suggesting that they may be DOR/MOR heteromers.^{95–97} Overall, the data are consistent with the hypothesis that morphine induces a population of pronociceptive DORs, which may include DOR1s and/or DOR/MOR heteromers. If this is the case, it should be possible to develop opioid drugs that selectively antagonize these receptors. Unbiased screening of diversified chemical libraries may hold the greatest promise for identifying highly selective heteromer ligands, as drug binding sites in heteromers may be wholly distinct from their constituent monomers.⁷¹ However, bivalent ligands that are composed of 2 covalently linked pharmacophores, which are each selective for a distinct monomer, offer a relatively straightforward and inexpensive way to identify and validate heteromer drug targets.⁹⁸ We are consequently in the process of designing and synthesizing such bivalent ligands with agonist, antagonist, and mixed agonist/antagonist properties at DOR/MOR heteromers.

Conclusion

Advances in clinical opioid pharmacology have been grossly incommensurate with the decades of effort and billions of dollars invested in basic research. Since the discovery of morphine over 200 years ago, faster acting and longer acting opioids have come on the market; however, for patients suffering with chronic pain, the therapeutic utility of all of these drugs is short-lived because of the rapid development of tolerance and withdrawal-induced hyperalgesia. Many physicians and patients are also afraid to begin treatment with opioids or discontinue treatment because of the development of physiological dependence and true psychological addiction. Some degree of tolerance and dependence may be inevitable with prolonged use of high doses of opioids; however, comparative studies of existing small molecule and peptide agonists suggest that it should be possible to design an opioid that retains the analgesic properties of existing drugs while minimizing their propensity to induce tolerance and dependence.

The MOR has been exceptionally well validated as a drug target for acute pain. However, if chronic pain is to be successfully treated, drug developers must look beyond efficacy and potency and consider additional properties that affect net receptor signaling, including receptor trafficking and signaling through noncanonical pathways such as arrestin. Our findings with mutant RMOR mice and morphine/methadone cocktails suggest that the ideal drug would recruit arrestin to the MOR and induce receptor endocytosis and recycling similarly to endogenous opioids. In addition, it is overly simplistic to screen drugs against the MOR in isolation, as research from our lab and others suggests that the endogenous receptor forms heteromers with other opioid receptors and possibly other GPCR families as well. The ideal drug might, for example, activate MOR homomers but spare DOR/MOR heteromers.

The goal of our research is to help guide the selection and development of a new generation of safer, more effective opioid drugs. Our findings have led us to make specific predictions regarding the desirable pharmacological qualities of such drugs. Although these qualities may continue to be debated in the scientific community for years to come, the clinic will be their true testing ground.

Acknowledgments

This work was supported by the National Institute on Drug Abuse (grants DA015232 and DA019958, J.L.W.) and funds provided by the state of California for medical research on alcohol and substance abuse through the University of California, San Francisco (J.L.W.).

Appendix

Opioids in Clinical Use

We predict that the ideal opioid would be highly selective for the MOR, have pharmacokinetic properties similar to morphine, but promote MOR desensitization and trafficking similar to endogenous opioids. Unfortunately, among the opioids commonly used to treat pain, no such drug currently exists.

Morphine and Codeine

These 2 drugs are the primary active ingredients of raw opium. Codeine is a prodrug that is converted in vivo into codeine-6-glucuronide and morphine, through which it exerts the majority of its analgesic effect. Compared to endogenous opioid peptides, both codeine and morphine induce significantly less MOR internalization in vitro and in vivo even when they are administered at higher doses for an extended period of time.⁸

Hydromorphone, Hydrocodone, Oxycodone, and Oxymorphone

Along with morphine, these drugs are the most commonly prescribed to chronic pain patients. They are all semisynthetic drugs that are derived from naturally occurring opiate alkaloids, such as morphine, and structurally, they are very similar to morphine. It is not surprising then that, like morphine, they are poor internalizers of the MOR,¹¹ and their use is associated with the development of both substantial tolerance and dependence.

Fentanyl

Fentanyl is a fully synthetic agonist that is structurally unrelated to morphine. It is commonly used as an induction agent for surgical anesthesia and, in transdermal and buccal preparations, as a treatment for chronic pain, particularly cancer pain. Fentanyl is both highly efficacious and highly potent and produces maximal analgesic effect at low receptor occupancy. Compared to morphine, it also induces a greater degree of MOR desensitization and endocytosis when applied at saturating concentrations in vitro.^{8,11} However, the dose of an agonist that is required for signal transduction is much lower than that required for receptor internalization,¹⁰ and at clinically relevant doses, fentanyl does not drive substantial MOR endocytosis. Fentanyl-occupied receptors would thus be expected to be persistently desensitized in some regions of the brain and persistently activated in others. Because fentanyl is able to drive strong signal transduction while occupying only a small percentage of the total receptor pool, we would predict that persistent MOR desensitization would not cause significant tolerance to fentanyl, but persistent activation would trigger compensatory changes that, in turn, would cause dependence and tolerance to other opioid agonists. Indeed, fentanyl is often effective in otherwise opioid-tolerant patients; however, its use is also associated with the rapid

development of hyperalgesia and an increase in the need for other opioid analgesics, frequently after even a single exposure to fentanyl.^{99,100}

Methadone

Methadone has a potency similar to morphine but, in contrast, is quite effective at stimulating receptor endocytosis.¹¹ However, methadone has a very long half-life, and unfortunately, there is a great deal of both pharmacokinetic and pharmacodynamic variability in methadone metabolism and response across the human population.¹⁰¹ In particular, in many patients, the effective half-life of methadone for analgesia does not mirror the half-life for respiratory suppression and cardiac side effects, making methadone difficult to dose consistently and safely. Therefore, methadone is only rarely used as a first line therapy for chronic pain. However, because it does facilitate MOR trafficking, we would predict that methadone would be able to overcome the portion of tolerance to other opioids that is due to persistent receptor desensitization rather than compensatory changes in signal transduction. Consistent with this, cross-tolerance to methadone is often incomplete, making methadone a common choice for opioid rotation in chronic pain patients.¹⁰²

References

1. Brownstein MJ. A brief history of opiates, opioid peptides, and opioid receptors. *Proc Natl Acad Sci U S A* 1993;90:5391–5393. [PubMed: 8390660]
2. Matthes HW, Maldonado R, Simonin F, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* 1996;383:819–823. [PubMed: 8893006]
3. Ferguson SS, Zhang J, Barak LS, Caron MG. Molecular mechanisms of G protein-coupled receptor desensitization and resensitization. *Life Sci* 1998;62:1561–1565. [PubMed: 9585136]
4. Keith DE, Murray SR, Zaki PA, et al. Morphine activates opioid receptors without causing their rapid internalization. *J Biol Chem* 1996;271:19021–19024. [PubMed: 8702570]
5. Arden JR, Segredo V, Wang Z, et al. Phosphorylation and agonist-specific intracellular trafficking of an epitope-tagged mu-opioid receptor expressed in HEK 293 cells. *J Neurochem* 1995;65:1636–1645. [PubMed: 7561859]
6. He L, Fong J, von Zastrow M, Whistler JL. Regulation of opioid receptor trafficking and morphine tolerance by receptor oligomerization. *Cell* 2002;108:271–282. [PubMed: 11832216]
7. He L, Whistler JL. An opiate cocktail that reduces morphine tolerance and dependence. *Curr Biol* 2005;15:1028–1033. [PubMed: 15936273]
8. Keith DE, Anton B, Murray SR, et al. mu-Opioid receptor internalization: opiate drugs have differential effects on a conserved endocytic mechanism in vitro and in the mammalian brain. *Mol Pharmacol* 1998;53:377–384. [PubMed: 9495801]
9. Sternini C, Spann M, Anton B, et al. Agonist-selective endocytosis of mu opioid receptor by neurons in vivo. *Proc Natl Acad Sci U S A* 1996;93:9241–9246. [PubMed: 8799185]
10. Alvarez VA, Arttamangkul S, Dang V, et al. mu-Opioid receptors: Ligand-dependent activation of potassium conductance, desensitization, and internalization. *J Neurosci* 2002;22:5769–5776. [PubMed: 12097530]
11. Koch T, Widera A, Bartsch K, et al. Receptor endocytosis counteracts the development of opioid tolerance. *Mol Pharmacol* 2005;67:280–287. [PubMed: 15475572]
12. Whistler JL, von Zastrow M. Morphine-activated opioid receptors elude desensitization by beta-arrestin. *Proc Natl Acad Sci U S A* 1998;95:9914–9919. [PubMed: 9707575]
13. Zhang J, Ferguson SS, Barak LS, et al. Role for G protein-coupled receptor kinase in agonist-specific regulation of mu-opioid receptor responsiveness. *Proc Natl Acad Sci U S A* 1998;95:7157–7162. [PubMed: 9618555]
14. Bailey CP, Kelly E, Henderson G. Protein kinase C activation enhances morphine-induced rapid desensitization of mu-opioid receptors in mature rat locus ceruleus neurons. *Mol Pharmacol* 2004;66:1592–1598. [PubMed: 15361548]

15. Bohn LM, Lefkowitz RJ, Caron MG. Differential mechanisms of morphine antinociceptive tolerance revealed in (beta)arrestin-2 knock-out mice. *J Neurosci* 2002;22:10494–10500. [PubMed: 12451149]
16. Bagley EE, Chieng BC, Christie MJ, Connor M. Opioid tolerance in periaqueductal gray neurons isolated from mice chronically treated with morphine. *Br J Pharmacol* 2005;146:68–76. [PubMed: 15980868]
17. Dang VC, Williams JT. Morphine-Induced mu-opioid receptor desensitization. *Mol Pharmacol* 2005;68:1127–1132. [PubMed: 16020743]
18. Johnson EE, Chieng B, Napier I, Connor M. Decreased mu-opioid receptor signalling and a reduction in calcium current density in sensory neurons from chronically morphine-treated mice. *Br J Pharmacol* 2006;148:947–955. [PubMed: 16783402]
19. Sim LJ, Selley DE, Dworkin SI, Childers SR. Effects of chronic morphine administration on mu opioid receptor-stimulated [35S]GTPgammaS autoradiography in rat brain. *J Neurosci* 1996;16:2684–2692. [PubMed: 8786444]
20. Connor M, Borgland SL, Christie MJ. Continued morphine modulation of calcium channel currents in acutely isolated locus coeruleus neurons from morphine-dependent rats. *Br J Pharmacol* 1999;128:1561–1569. [PubMed: 10602337]
21. Blanchet C, Luscher C. Desensitization of mu-opioid receptor-evoked potassium currents: initiation at the receptor, expression at the effector. *Proc Natl Acad Sci U S A* 2002;99:4674–4679. [PubMed: 11917119]
22. Ingram SL, Vaughan CW, Bagley EE, et al. Enhanced opioid efficacy in opioid dependence is caused by an altered signal transduction pathway. *J Neurosci* 1998;18:10269–10276. [PubMed: 9852564]
23. Terman GW, Jin W, Cheong YP, et al. G-protein receptor kinase 3 (GRK3) influences opioid analgesic tolerance but not opioid withdrawal. *Br J Pharmacol* 2004;141:55–64. [PubMed: 14662727]
24. Bohn LM, Lefkowitz RJ, Gainetdinov RR, et al. Enhanced morphine analgesia in mice lacking beta-arrestin 2. *Science* 1999;286:2495–2498. [PubMed: 10617462]
25. Bohn LM, Gainetdinov RR, Lin FT, et al. Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. *Nature* 2000;408:720–723. [PubMed: 11130073]
26. Bohn LM, Dykstra LA, Lefkowitz RJ, et al. Relative opioid efficacy is determined by the complements of the G protein-coupled receptor desensitization machinery. *Mol Pharmacol* 2004;66:106–112. [PubMed: 15213301]
27. Virk MS, Williams JT. Agonist-specific regulation of mu-opioid receptor desensitization and recovery from desensitization. *Mol Pharmacol* 2008;73:1301–1308. [PubMed: 18198283]
28. Johnson EA, Oldfield S, Braksator E, et al. Agonist-selective mechanisms of mu-opioid receptor desensitization in human embryonic kidney 293 cells. *Mol Pharmacol* 2006;70:676–685. [PubMed: 16682505]
29. Chu J, Zheng H, Loh HH, Law PY. Morphine-induced mu-opioid receptor rapid desensitization is independent of receptor phosphorylation and beta-arrestins. *Cell Signal* 2008;20:1616–1624. [PubMed: 18558479]
30. Smith FL, Lohmann AB, Dewey WL. Involvement of phospholipid signal transduction pathways in morphine tolerance in mice. *Br J Pharmacol* 1999;128:220–226. [PubMed: 10498855]
31. Smith FL, Javed R, Elzey MJ, et al. Prolonged reversal of morphine tolerance with no reversal of dependence by protein kinase C inhibitors. *Brain Res* 2002;958:28–35. [PubMed: 12468027]
32. Esmacili-Mahani S, Shimokawa N, Javan M, et al. Low-dose morphine induces hyperalgesia through activation of G alphas, protein kinase C, and L-type Ca²⁺ channels in rats. *J Neurosci Res* 2008;86:471–479. [PubMed: 17893922]
33. Galeotti N, Stefano GB, Guarna M, et al. Signaling pathway of morphine induced acute thermal hyperalgesia in mice. *Pain* 2006;123:294–305. [PubMed: 16650582]
34. Ueda H, Inoue M, Matsumoto T. Protein kinase C-mediated inhibition of mu-opioid receptor internalization and its involvement in the development of acute tolerance to peripheral mu-agonist analgesia. *J Neurosci* 2001;21:2967–2973. [PubMed: 11312280]
35. Zheng H, Chu J, Qiu Y, et al. Agonist-selective signaling is determined by the receptor location within the membrane domains. *Proc Natl Acad Sci U S A* 2008;105:9421–9426. [PubMed: 18599439]

36. Monastyrskaya K, Hostettler A, Buergi S, Draeger A. The NK1 receptor localizes to the plasma membrane microdomains, and its activation is dependent on lipid raft integrity. *J Biol Chem* 2005;280:7135–7146. [PubMed: 15590676]
37. Shih M, Malbon CC. Oligodeoxynucleotides antisense to mRNA encoding protein kinase A, protein kinase C, and beta-adrenergic receptor kinase reveal distinctive cell-type-specific roles in agonist-induced desensitization. *Proc Natl Acad Sci U S A* 1994;91:12193–12197. [PubMed: 7991605]
38. Shih M, Malbon CC. Protein kinase C deficiency blocks recovery from agonist-induced desensitization. *J Biol Chem* 1996;271:21478–21483. [PubMed: 8702931]
39. Gabra BH, Bailey CP, Kelly E, et al. Evidence for an important role of protein phosphatases in the mechanism of morphine tolerance. *Brain Res* 2007;1159:86–93. [PubMed: 17582387]
40. Kim JA, Bartlett S, He L, et al. Morphine-induced receptor endocytosis in a novel knockin mouse reduces tolerance and dependence. *Curr Biol* 2008;18:129–135. [PubMed: 18207746]
41. King T, Ossipov MH, Vanderah TW, et al. Is paradoxical pain induced by sustained opioid exposure an underlying mechanism of opioid antinociceptive tolerance? *Neurosignals* 2005;14:194–205. [PubMed: 16215302]
42. Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. *Physiol Rev* 2001;81:299–343. [PubMed: 11152760]
43. Nestler EJ. Under siege: the brain on opiates. *Neuron* 1996;16:897–900. [PubMed: 8630245]
44. Lane-Ladd SB, Pineda J, Boundy VA, et al. CREB (cAMP response element-binding protein) in the locus coeruleus: biochemical, physiological, and behavioral evidence for a role in opiate dependence. *J Neurosci* 1997;17:7890–7901. [PubMed: 9315909]
45. Hack SP, Vaughan CW, Christie MJ. Modulation of GABA release during morphine withdrawal in midbrain neurons in vitro. *Neuropharmacology* 2003;45:575–584. [PubMed: 12941371]
46. Chieng B, Williams JT. Increased opioid inhibition of GABA release in nucleus accumbens during morphine withdrawal. *J Neurosci* 1998;18:7033–7039. [PubMed: 9712672]
47. Bonci A, Williams JT. Increased probability of GABA release during withdrawal from morphine. *J Neurosci* 1997;17:796–803. [PubMed: 8987801]
48. Koob GF, Maldonado R, Stinus L. Neural substrates of opiate withdrawal. *Trends Neurosci* 1992;15:186–191. [PubMed: 1377426]
49. Stinus L, Le Moal M, Koob GF. Nucleus accumbens and amygdala are possible substrates for the aversive stimulus effects of opiate withdrawal. *Neuroscience* 1990;37:767–773. [PubMed: 2247222]
50. Maldonado R, Stinus L, Gold LH, Koob GF. Role of different brain structures in the expression of the physical morphine withdrawal syndrome. *J Pharmacol Exp Ther* 1992;261:669–677. [PubMed: 1578378]
51. Punch LJ, Self DW, Nestler EJ, Taylor JR. Opposite modulation of opiate withdrawal behaviors on microinfusion of a protein kinase A inhibitor versus activator into the locus coeruleus or periaqueductal gray. *J Neurosci* 1997;17:8520–8527. [PubMed: 9334424]
52. Ma J, Pan ZZ. Contribution of brainstem GABA(A) synaptic transmission to morphine analgesic tolerance. *Pain* 2006;122:163–173. [PubMed: 16527406]
53. Grecksch G, Bartzsch K, Widera A, et al. Development of tolerance and sensitization to different opioid agonists in rats. *Psychopharmacology (Berl)* 2006;186:177–184. [PubMed: 16572262]
54. Duttaroy A, Yoburn BC. The effect of intrinsic efficacy on opioid tolerance. *Anesthesiology* 1995;82:1226–1236. [PubMed: 7741298]
55. Walker EA, Young AM. Differential tolerance to antinociceptive effects of mu opioids during repeated treatment with etonitazene, morphine, or buprenorphine in rats. *Psychopharmacology (Berl)* 2001;154:131–142. [PubMed: 11314675]
56. Finn AK, Whistler JL. Endocytosis of the mu opioid receptor reduces tolerance and a cellular hallmark of opiate withdrawal. *Neuron* 2001;32:829–839. [PubMed: 11738029]
57. He L, Kim JA, Whistler JL. Biomarkers of morphine tolerance and dependence are prevented by morphine-induced endocytosis of a mutant mu-opioid receptor. *FASEB J* 2009;23:4327–4334. [PubMed: 19679639]

58. Madhavan, A.; He, L.; Stuber, GD., et al. Morphine-induced mu opioid receptor endocytosis prevents adaptations in GABA release during withdrawal. International Narcotics Research Conference; Portland, OR. July 12–17, 2009;
59. Ravindranathan A, Joslyn G, Robertson M, et al. Functional characterization of human variants of the mu-opioid receptor gene. *Proc Natl Acad Sci U S A* 2009;106:10811–10816. [PubMed: 19528663]
60. Wang D, Sun X, Bohn LM, Sadee W. Opioid receptor homo- and heterodimerization in living cells by quantitative bioluminescence resonance energy transfer. *Mol Pharmacol* 2005;67:2173–2184. [PubMed: 15778451]
61. Hojo M, Sudo Y, Ando Y, et al. mu-Opioid receptor forms a functional heterodimer with cannabinoid CB1 receptor: electro-physiological and FRET assay analysis. *J Pharmacol Sci* 2008;108:308–319. [PubMed: 19008645]
62. Pfeiffer M, Kirscht S, Stumm R, et al. Heterodimerization of substance P and mu-opioid receptors regulates receptor trafficking and resensitization. *J Biol Chem* 2003;278:51630–51637. [PubMed: 14532289]
63. Lopez-Gimenez JF, Vilaro MT, Milligan G. Morphine desensitization, internalization, and down-regulation of the mu opioid receptor is facilitated by serotonin 5-hydroxytryptamine2A receptor coactivation. *Mol Pharmacol* 2008;74:1278–1291. [PubMed: 18703670]
64. He L, Whistler JL. The biochemical analysis of methadone modulation on morphine-induced tolerance and dependence in the rat brain. *Pharmacology* 2007;79:193–202. [PubMed: 17356311]
65. Hashimoto T, Saito Y, Yamada K, et al. Enhancement of morphine analgesic effect with induction of mu-opioid receptor endocytosis in rats. *Anesthesiology* 2006;105:574–580. [PubMed: 16931992]
66. Zollner C, Mousa SA, Fischer O, et al. Chronic morphine use does not induce peripheral tolerance in a rat model of inflammatory pain. *J Clin Invest* 2008;118:1065–1073. [PubMed: 18246198]
67. Jordan BA, Devi LA. G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* 1999;399:697–700. [PubMed: 10385123]
68. George SR, Fan T, Xie Z, et al. Oligomerization of mu- and delta-opioid receptors. Generation of novel functional properties. *J Biol Chem* 2000;275:26128–26135. [PubMed: 10842167]
69. Gomes I, Gupta A, Filipovska J, et al. A role for heterodimerization of mu and delta opiate receptors in enhancing morphine analgesia. *Proc Natl Acad Sci U S A* 2004;101:5135–5139. [PubMed: 15044695]
70. Gomes I, Jordan BA, Gupta A, et al. Heterodimerization of mu and delta opioid receptors: a role in opiate synergy. *J Neurosci* 2000;20:RC110. [PubMed: 11069979]
71. Waldhoer M, Fong J, Jones RM, et al. A heterodimer-selective agonist shows in vivo relevance of G protein-coupled receptor dimers. *Proc Natl Acad Sci U S A* 2005;102:9050–9055. [PubMed: 15932946]
72. Cahill CM, Morinville A, Lee MC, et al. Prolonged morphine treatment targets delta opioid receptors to neuronal plasma membranes and enhances delta-mediated antinociception. *J Neurosci* 2001;21:7598–7607. [PubMed: 11567050]
73. Guan JS, Xu ZZ, Gao H, et al. Interaction with vesicle luminal protachykinin regulates surface expression of delta-opioid receptors and opioid analgesia. *Cell* 2005;122:619–631. [PubMed: 16122428]
74. Hack SP, Bagley EE, Chieng BC, Christie MJ. Induction of delta-opioid receptor function in the midbrain after chronic morphine treatment. *J Neurosci* 2005;25:3192–3198. [PubMed: 15788776]
75. Ma J, Zhang Y, Kalyuzhny AE, Pan ZZ. Emergence of functional delta-opioid receptors induced by long-term treatment with morphine. *Mol Pharmacol* 2006;69:1137–1145. [PubMed: 16399848]
76. Zhu Y, King MA, Schuller AG, et al. Retention of supraspinal delta-like analgesia and loss of morphine tolerance in delta opioid receptor knockout mice. *Neuron* 1999;24:243–252. [PubMed: 10677041]
77. Kest B, Lee CE, McLemore GL, Inturrisi CE. An antisense oligodeoxynucleotide to the delta opioid receptor (DOR-1) inhibits morphine tolerance and acute dependence in mice. *Brain Res Bull* 1996;39:185–188. [PubMed: 8866695]

78. Nitsche JF, Schuller AG, King MA, et al. Genetic dissociation of opiate tolerance and physical dependence in delta-opioid receptor-1 and preproenkephalin knock-out mice. *J Neurosci* 2002;22:10906–10913. [PubMed: 12486185]
79. Chefer VI, Shippenberg TS. Augmentation of morphine-induced sensitization but reduction in morphine tolerance and reward in delta-opioid receptor knockout mice. *Neuropsychopharmacology* 2009;34:887–898. [PubMed: 18704097]
80. Marquez P, Baliram R, Gajawada N, et al. Differential involvement of enkephalins in analgesic tolerance, locomotor sensitization, and conditioned place preference induced by morphine. *Behav Neurosci* 2006;120:10–15. [PubMed: 16492112]
81. Abdelhamid EE, Sultana M, Portoghese PS, Takemori AE. Selective blockage of delta opioid receptors prevents the development of morphine tolerance and dependence in mice. *J Pharmacol Exp Ther* 1991;258:299–303. [PubMed: 1649297]
82. Roy S, Guo X, Kelschenbach J, et al. In vivo activation of a mutant mu-opioid receptor by naltrexone produces a potent analgesic effect but no tolerance: role of mu-receptor activation and delta-receptor blockade in morphine tolerance. *J Neurosci* 2005;25:3229–3233. [PubMed: 15788780]
83. Chaillet P, Coulaud A, Zajac JM, et al. The mu rather than the delta subtype of opioid receptors appears to be involved in enkephalin-induced analgesia. *Eur J Pharmacol* 1984;101:83–90. [PubMed: 6086366]
84. Porreca F, Mosberg HI, Hurst R, et al. Roles of mu, delta and kappa opioid receptors in spinal and supraspinal mediation of gastrointestinal transit effects and hot-plate analgesia in the mouse. *J Pharmacol Exp Ther* 1984;230:341–348. [PubMed: 6086883]
85. Riba P, Ben Y, Smith AP, et al. Morphine tolerance in spinal cord is due to interaction between mu- and delta-receptors. *J Pharmacol Exp Ther* 2002;300:265–272. [PubMed: 11752125]
86. Larson AA, Vaught JL, Takemori AE. The potentiation of spinal analgesia by leucine enkephalin. *Eur J Pharmacol* 1980;61:381–383. [PubMed: 6892802]
87. Qi JN, Mosberg HI, Porreca F. Modulation of the potency and efficacy of mu-mediated antinociception by delta agonists in the mouse. *J Pharmacol Exp Ther* 1990;254:683–689. [PubMed: 2166801]
88. Ossipov MH, Kovelowski CJ, Nichols ML, et al. Characterization of supraspinal antinociceptive actions of opioid delta agonists in the rat. *Pain* 1995;62:287–293. [PubMed: 8657428]
89. Qi JA, Mosberg HI, Porreca F. Antinociceptive effects of [D-Ala2]deltorphin II, a highly selective delta agonist in vivo. *Life Sci* 1990;47:PL43–PL47. [PubMed: 2170791]
90. Qi JA, Bowen WD, Mosberg HI, et al. Opioid agonist and antagonist antinociceptive properties of [D-Ala2,Leu5,Cys6]enkephalin: selective actions at the deltanoncomplexed site. *J Pharmacol Exp Ther* 1990;255:636–641. [PubMed: 2173752]
91. van Rijn RM, Whistler JL. The delta(1) opioid receptor is a heterodimer that opposes the actions of the delta(2) receptor on alcohol intake. *Biol Psychiatry* 2009;66:777–784. [PubMed: 19576572]
92. Matthes HW, Smadja C, Valverde O, et al. Activity of the delta-opioid receptor is partially reduced, whereas activity of the kappa-receptor is maintained in mice lacking the mu-receptor. *J Neurosci* 1998;18:7285–7295. [PubMed: 9736649]
93. Morinville A, Cahill CM, Esdaile MJ, et al. Regulation of delta-opioid receptor trafficking via mu-opioid receptor stimulation: evidence from mu-opioid receptor knock-out mice. *J Neurosci* 2003;23:4888–4898. [PubMed: 12832511]
94. Pradhan AA, Siau C, Constantin A, Clarke PB. Chronic morphine administration results in tolerance to delta opioid receptor-mediated antinociception. *Neuroscience* 2006;141:947–954. [PubMed: 16716526]
95. Abul-Husn NS, Sutak M, Milne B, Jhamandas K. Augmentation of spinal morphine analgesia and inhibition of tolerance by low doses of mu- and delta-opioid receptor antagonists. *Br J Pharmacol* 2007;151:877–887. [PubMed: 17502848]
96. Wang HY, Friedman E, Olmstead MC, Burns LH. Ultra-low-dose naloxone suppresses opioid tolerance, dependence and associated changes in mu opioid receptor-G protein coupling and Gbetagamma signaling. *Neuroscience* 2005;135:247–261. [PubMed: 16084657]
97. Crain SM, Shen KF. Ultra-low concentrations of naloxone selectively antagonize excitatory effects of morphine on sensory neurons, thereby increasing its antinociceptive potency and attenuating

- tolerance/dependence during chronic cotreatment. *Proc Natl Acad Sci U S A* 1995;92:10540–10544. [PubMed: 7479836]
98. Portoghese PS. From models to molecules: opioid receptor dimers, bivalent ligands, and selective opioid receptor probes. *J Med Chem* 2001;44:2259–2269. [PubMed: 11428919]
99. Angst MS, Koppert W, Pahl I, et al. Short-term infusion of the mu-opioid agonist remifentanyl in humans causes hyperalgesia during withdrawal. *Pain* 2003;106:49–57. [PubMed: 14581110]
100. Laulin JP, Maurette P, Corcuff JB, et al. The role of ketamine in preventing fentanyl-induced hyperalgesia and subsequent acute morphine tolerance. *Anesth Analg* 2002;94:1263–1269. [PubMed: 11973202]
101. Eap CB, Buclin T, Baumann P. Interindividual variability of the clinical pharmacokinetics of methadone: implications for the treatment of opioid dependence. *Clin Pharmacokinet* 2002;41:1153–1193. [PubMed: 12405865]
102. Mercadante S. Opioid rotation for cancer pain: rationale and clinical aspects. *Cancer* 1999;86:1856–1866. [PubMed: 10547561]

FIGURE 1.

Impairment of mu opioid receptor (MOR) trafficking in response to morphine contributes to tolerance and dependence. (A) Normal cycling of the MOR in response to endogenous enkephalin (ENK). Ligand binding activates signal transduction, leading to a reduction in cyclic adenosine monophosphate (cAMP). Activated receptors are rapidly silenced by receptor phosphorylation and arrestin (arr) binding. Subsequently, agonist responsiveness is restored by receptor endocytosis and recycling to the plasma membrane. Signal transduction in response to enkephalin is therefore pulsatile: on-off-on-off. (B) Abnormal cycling of the MOR in response to morphine (MOR). Morphine induces desensitization in some brain regions but not others and, compared to enkephalin, stimulates very little receptor endocytosis. In brain regions

where morphine induces neither desensitization nor endocytosis, signal transduction is persistently on, leading to homeostatic counteradaptations that manifest as tolerance and dependence. In brain regions where morphine induces desensitization without endocytosis, signal transduction is persistently off, leading to a prolonged loss of agonist responsiveness that contributes to tolerance but not dependence. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIGURE 2.

A cocktail of morphine and methadone promotes less tolerance and dependence than morphine alone. Rats were implanted with intraventricular cannulae and given twice daily infusions of opioids for 5 days. (A) Tolerance. Rats treated with an analgesic dose of morphine (30nmol) developed substantial tolerance over the course of 5 days (*black circles*). In contrast, rats treated with the same dose of morphine combined with methadone (50nmol) did not develop significant tolerance (*red squares*), although the amount of methadone used was insufficient to produce analgesia on its own (*gray triangles*). The dose of methadone was chosen for its ability to facilitate endocytosis of morphine-bound mu opioid receptors. (B) Dependence. After their final infusion of opioid agonists, rats were injected with subcutaneous naloxone (3mg/

kg) to precipitate withdrawal. Rats treated with the morphine/methadone (Mor/Meth) cocktail (*red bar*) exhibited significantly fewer withdrawal signs than rats treated with morphine alone (*black bar*). MPE = maximum possible effect. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]