Themed Issue: Drug Addiction - From Basic Research to Therapies
Guest Editors - Rao Rapaka and Wolfgang Sadée

Mu Opioid Receptor Regulation And Opiate Responsiveness

Submitted: April 22, 2005; Accepted: April 27, 2005; Published: October 19, 2005

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ABSTRACT

Opiate drugs such as morphine are well known for their ability to produce potent analgesia as well as such unwanted side effects as tolerance, physical dependence, respiratory suppression and constipation. Opiates act at opioid receptors, which belong to the family of G protein-coupled receptors. The mechanisms governing mu opioid receptor (μOR) regulation are of particular interest since morphine and other clinically important analgesics produce their pharmacological effects through this receptor. Here we review recent advances in understanding how opioid receptor regulation can impart differential agonist efficacy produced in vivo.

INTRODUCTION

Of the three major classes of opioid receptors, mu (μ), delta (δ), and kappa (κ), the μOR has proven to be the major target of opiate analgesics (for reviews see[1-3]). The opioid receptors belong to the family of G protein-coupled receptors (GPCRs), and like most GPCRs, they can be regulated by multiple mechanisms including receptor desensitization, internalization, resensitization and downregulation. G protein-coupled receptor regulatory elements such as GPCR kinases (GRKs) and βarrestins are important mediators of these processes. Agonist stimulation of GPCRs promotes receptor phosphorylation by GRKs and leads to recruitment of βarrestins which effectively uncouple the receptor and G proteins, thus preventing further signaling.[4-6] In addition to mediating receptor desensitization, βarrestins also facilitate the internalization of inactivated receptors which can promote receptor recycling to the plasma membrane or lead to downregulation by receptor degradation.[4-6] βarrestins were first described for their ability to negatively regulate GPCR signaling (ie, desensitization).[7,8] However, βarrestins can also play a more complex role in mediating receptor signaling and increasing evidence suggests that the complement of certain scaffolding proteins within the cellular environment may play a major role in determining overall receptor responsiveness to different agonists in particular cell types.[5,6]

The role of βarrestins in regulating the μOR has been studied at both the molecular level in vitro and at the pharmacological level in vivo (for reviews see[9-12]). Early in vitro studies in transfected HEK-293 cells revealed that the μOR, upon activation with morphine, does not robustly recruit βarrestins to the membrane while other opioid agonists, such as etorphine, do.[13] Since agonist activation of GPCRs typically induces βarrestin recruitment, morphine's actions at the μOR are unusual. These early observations suggested that the morphine-bound μOR may not be regulated by βarrestins. However, the physiological importance of μOR-βarrestin interactions was soon revealed when morphine-induced behaviors were evaluated in mice lacking βarrestin2.

Mice lacking βarrestin2 appear normal, although this molecule has been implicated in regulating numerous GPCRs that are expressed throughout the body. When morphine is administered to these animals, striking differences become immediately apparent when they are compared with normal, wild-type (WT) mice. βarrestin2-knockout (βarr2-KO) mice display enhanced and prolonged morphine-induced analgesia in both hot-plate and tail-flick antinociceptive tests.[14,15] Moreover, morphine-induced striatal extracellular dopamine levels as well as drug reinforcement are enhanced in the βarr2-KO mice compared with their WT counterparts.[16] Further investigation into behaviors in the absence of drug, revealed that basal tail-flick nociceptive response latencies are prolonged and this effect can be blocked by the opiate antagonist, naltrexone.[15] This suggests that the μOR-βarrestin2 interaction may not only be important for regulating the morphine-activated receptor, but may also help to establish the basal tone of receptor signaling. This finding also correlates with the observation that μOR agonist stimulated G protein-coupling is elevated in βarr2-KO mouse brain regions (periaqueductal gray, brainstem) as well as spinal cord.[14,15,17] In the absence of agonist stimulation, the basal degree of μOR-G protein-coupling is also significantly higher in brain regions in βarr2-KO compared with WT mice (LM Bohn, D Wang, W Sadée, unpublished observations). Therefore, the role of βarrestin2 in regulating the μOR is important for setting the basal tone as well as determining the potential for agonist-activated receptor signaling.

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In the presence of persistent agonist treatment, GPCRs are subject to desensitization. Chronic morphine treatment, in vivo, leads to the development of opiate antinociceptive tolerance and physical dependence. Antinociceptive tolerance has previously been correlated with μOR desensitization[18,19] yet this has been difficult to test experimentally since there are no pharmacological tools which directly block desensitization. The βarr2-KO mice, after several different regimens of chronic morphine treatment, do not develop morphine-induced tolerance in the hot plate test, and display greatly attenuated tolerance in the tail-flick test.[15,17] Moreover, G protein-coupling in periaqueductal gray and brainstem of mice chronically treated with morphine reveal that while the μOR is significantly uncoupled from G proteins in WT mice, coupling is preserved in the βarr2-KO mice.[17] In another set of studies, Przewlocka et al.[20] showed that intrathecal administration of βarrestin2-specific antisense oligonucleotides could delay the onset of morphine antinociceptive tolerance in mice. Taken together, the biochemical and behavioral data suggest that βarrestin2 acts as a negative regulator, or desensitizing component, of μOR signaling in vivo.

While many of the morphine-induced responses in the βarr2-KO mice support the classically defined role of βarrestins as negative regulators of GPCR signaling, other physiological and behavioral responses to morphine do not. Morphine is known to activate locomotor activity in mice; however, the βarr2-KO mice display *less* activation of locomotion compared with their WT counterparts despite increased extracellular dopamine levels in striatum.[16] Moreover, while the βarr2-KO mice are resistant to morphine-induced antinociceptive tolerance, both genotypes develop a similar extent of physical dependence.[17] Current studies of respiratory suppression and gastrointestinal transit suggest that morphine-induced side effects are also not enhanced and may be less severe in mice lacking βarrestin2[21]

The question arises as to whether βarrestin2 may also be playing a role as a positive mediator of μOR signaling in vivo. Although βarrestins are traditionally viewed as negative regulators of GPCR signaling, βarrestins also function as scaffolding molecules that mediate GPCR signaling by facilitating interactions between signaling proteins and the receptor. In this scenario, μOR signaling may differ in certain cell types wherein the receptor's fate may be determined by the cellular complement of proteins within the receptor's immediate environment. Several in vitro studies have demonstrated that βarrestins act as adaptors between GPCRs and intracellular signaling proteins including the non-receptor tyrosine kinase, c-Src,[22-28] extracellular signal-regulated kinases (ERK)[22,25,29-32] and c-Jun N-terminal kinase (JNK).[33] The role of βarrestin2 in modulating receptor signaling in vivo has been demonstrated by a recent study by Wang et al.[34] wherein βarr2-KO mice developed *less* sedation with the alpha adrenergic 2A receptor agonist UK 14,304 in the rotorod test, suggesting that βarrestin2 may be directly involved in promoting this response rather then attenuating it.

The unbiquitination of βarrestins is yet another mechanism that plays a role in regulating βarrestin-mediated internalization and/or signaling via GPCRs. Agonist-stimulated ubiquitination of βarrestin2 has been implicated in co-trafficking and subsequent endocytosis of several GPCRs.[35-37] The ubiquitinated receptor-βarrestin complex may also be important for initiating βarrestin-mediated signal transduction wherein endosomes containing receptor-βarrestin complexes may act as 'signalsomes' by promoting receptor endocytosis as well as G-protein independent signaling.[37] However, such a role for βarrestins has yet to be demonstrated in μOR signaling.

It is apparent that the current understanding of GPCR signaling is rapidly expanding past the classical models of G-protein coupling and βarrestin-mediated desensitization. The complexity of determining receptor conformation, signaling and regulation is compounded by the organization of GPCRs into dimers and multimers. Interactions between receptors, as homo-, hetero- or oligo-mers, could change receptor expression profiles, ligand binding, and receptor signaling as well as trafficking and regulation. Cvejic and Devi[38] reported that δ opioid receptors (δORs) can exist as dimers in vitro and that the dimer complex can be desensitized in an agonist-dependent manner. Heterodimerization between δ- and κORs confers different receptor properties with distinct binding and signal transduction profiles compared with either the κ- or δOR alone.[39] The μ- and δORs can heterodimerize and, in the presence of δ-antagonists, μOR agonist binding and signaling is enhanced.[40] This finding was extended to animals wherein δ-antagonists significantly augmented morphine-induced analgesia in mice.[40] Recently, Wang et al.[41] demonstrated that all three opioid receptors (μ, δ, κ) have an equal potential to form homo- or heterodimers with each other. Interactions between opioid receptors and other receptor types including the β_2-adrenergic,[42] nociceptin/orphanin FQ,[43] somatostatin receptors[44,45] and substance P receptors[46] have been reported in vitro and may further increase the level of complexity in conferring opioid receptor responsiveness.

Signaling via the μOR, therefore, has the potential to be regulated by multiple means. Even if the μOR is regulated by the classical desensitization paradigm by βarrestin2 in some neurons this may not hold true for other cell types. For example, the μOR is widely distributed throughout the CNS and periphery and therefore, μORs expressed in one particular cell type (i.e medium spiny neurons) may not be subject to the same regulatory mechanisms as μORs expressed in other cell types (ie, enteric neurons). Studies have shown decreased μOR-G protein coupling following morphine treatment in several

brainstem regions of rat including the dorsal raphe nucleus, locus coeruleus, parabrachial nuclei, and the commissural nucleus tractus solitatius while no changes in μOR-G protein-coupling were observed in other regions such as the nucleus accumbens, amygdala, thalamus, and substania nigra.[19] Decreases in μOR activated G protein-coupling in the same regions affected by morphine (periaqueductal gray, locus coeruleus, and lateral parabrachial nucleus) were also seen in rats self-administering heroin.[47] Further, chronic morphine has been shown to induce desensitization of the μOR as measured by adenylyl cyclase inhibition in thalamus and periaqueductal gray brain regions but not in caudate putamen or nucleus accumbens.[18] These observations suggest that while the μOR is expressed in these brain regions, it is not desensitized to the same extent following chronic morphine treatment and demonstrates that the μOR can be differentially regulated in different cellular environments.

The relative responsiveness of the μOR is not only dependent on agonist occupancy but can vary with distinct opiate agonists. Several groups have demonstrated in vitro that while agonists such as morphine, DAMGO, etorphine, methadone and fentanyl can activate μOR signaling with similar efficacy they differ in their ability to promote receptor desensitization and internalization.[48-50] For example, morphine and heroin do not promote robust βarrestin2 translocation or receptor endocytosis in HEK-293 cells while other opiate agonists including DAMGO, etorphine, methadone and fentanyl do.[13,50-55] The inability of morphine and heroin to induce βarrestin2 translocation could however be overcome by the overexpression of GRK2.[13,54] Studies in mouse embryonic fibroblasts lacking endogenous βarrestin1 and βarrestin2 suggest that the morphine-bound μOR preferentially interacts with βarrestin2.[54] This concept is further strengthened by the finding that the enhanced morphine analgesia in βarr2-KO mice could not be recapitulated in mice lacking βarrestin1, indicating that βarrestin2, rather than βarrestin1, may preferentially regulate the μOR in vivo.[54] Cheng et al.[56] showed that βarrestin1 interferes with δ- and κOR stimulated G protein-coupling but had no effect on μOR activation of G proteins further supporting a selective interaction between the μOR and βarrestin2 rather than βarrestin1.

Studies in cell culture reveal that the morphine-bound μOR is weakly phosphorylated, a poor substrate for βarrestins, and does not internalize. However, the overexpression of βarrestins or GRKs can overcome these apparent limitations.[13,53,54] Therefore, it is reasonable that if a certain neuron expresses higher levels of βarrestins or GRKs, the μOR may be able to internalize with morphine binding. While studies have nicely shown different levels of GRK and βarrestin mRNA expression in certain brain regions,[57] a lack of selective antibody tools have made it difficult to quantify protein expression patterns of each GRK and βarrestin type. Furthermore, GRK and βarrestin levels are dynamic and opiate agonists have been shown to alter their expression patterns throughout the CNS. Terwilliger et al.[58] reported that βarrestin1 and βarrestin2 levels increase in locus coeruleus neurons in response to chronic morphine treatment. In addition, acute and chronic morphine treatment also differentially alters βarrestin1 and βarrestin2 mRNA expression patterns in hippocampal, cerebral cortex, periaqueductal gray and locus coeruleus.[59] Mice acutely or chronically treated with the opiate agonist sufentanil have upregulated GRK2, GRK6 and βarrestin2 levels in brain while GRK3 levels are only elevated after acute treatment.[60] Increased levels of GRK2, GRK3, GRK6 and βarrestin2 in the cortex and striatum have also been observed following chronic opioid antagonist treatment with naloxone and naltrexone.[61] Finally, decreases in μOR density as well as GRK2, GRK6 and βarrestin2 levels in the prefrontal cortex have been observed in post-mortem brains of opiate addicts.[62]

Overall, there is a great deal of evidence supporting the dynamic expression of GRKs and βarrestins in the central nervous system. Therefore, μOR regulation profiles may also be dynamic, dependent not only on the site of expression but also upon drug exposure. Recently, Haberstock-Debic et al.[63] reported that while morphine-bound μORs do not internalize in the cell body of neurons, receptor internalization does occur in the dendrites of the same hippocampal neuron. This observation further emphasizes and points to the importance of the immediate cellular environment to the overall receptor regulation. Upon considering both the cell culture and animal studies in parallel, it is apparent that opioid receptor regulation can have profound impacts on overall agonist responsiveness. The complexity governing such diverse potential regulatory mechanisms emphasizes the need to study receptor signaling in the endogenous environment as this may ultimately determine the physiological response to the drug. As these complexities are revealed, novel therapeutic targets may become available to enhance and fine-tune opioid receptor pharmacology for the treatment of pain and addiction.

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