Molecular Physiology of Enteric Opioid Receptors

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Abstract

Opioid drugs have powerful antidiarrheal effects and many patients taking these drugs for chronic pain relief experience chronic constipation that can progress to opioid-induced bowel dysfunction. Three classes of opioid receptors are expressed by enteric neurons: μ-, δ-, and κ-opioid receptors (MOR, DOR, and KOR). MOR and DOR couple to inhibition of adenylate cyclase and nerve terminal Ca\(^{2+}\) channels and activation of K\(^{+}\) channels. These effects reduce neuronal activity and neurotransmitter release. KOR couples to inhibition of Ca\(^{2+}\) channels and inhibition of neurotransmitter release. In the human gastrointestinal tract, MOR, DOR, and KOR link to inhibition of acetylcholine release from enteric interneurons and purine/nitric oxide release from inhibitory motorneurons. These actions inhibit propulsive motility. MOR and DOR also link to inhibition of submucosal secretomotor neurons, reducing active Cl\(^{-}\) secretion and passive water movement into the colonic lumen. These effects account for the constipation caused by opioid receptor agonists. Tolerance develops to the analgesic effects of opioid receptor agonists but not to the constipating actions. This may be due to differential β-arrestin-2-dependent opioid receptor desensitization and internalization in enteric nerves in the colon compared with the small intestine and in neuronal pain pathways. Further studies of differential opioid receptor desensitization and tolerance in subsets of enteric neurons may identify new drugs or other treatment strategies of opioid-induced bowel dysfunction.

INTRODUCTION

Opiate drugs have powerful effects on gastrointestinal function, and chronic opiate use for pain relief can cause opioid-induced bowel dysfunction (chronic constipation) and, in
extreme cases, narcotic bowel syndrome (1). Other articles in this supplement will address the clinical issues surrounding opiate use and gut function. This review will focus on the physiology of opioid receptors in the gastrointestinal tract and the mechanisms by which opioids alter gut motility and secretion after acute and chronic treatment with opioid receptor agonist drugs.

**Opiate receptors in the enteric nervous system**

There are three classes of opioid receptors: μ, δ and κ, which are G-protein-coupled receptors (2). All three receptor subtypes are expressed in the gastrointestinal tract where they are localized principally to enteric neurons (3,4). Opioid receptor agonists produce constipation by inhibiting enteric neuron function. μ- and δ-opiate receptors (MOR and DOR) are expressed by enteric neurons and these receptors use common signaling pathways in the enteric nervous system (ENS). Both receptors link to the inhibitory G i G-protein to cause inhibition of adenylate cyclase, reduced cyclic 3′,5′-adenosine monophosphate, and reduced levels of protein kinase A activation (5,6). This results in reduced neuronal excitability. MOR and DOR also couple to the G o subtype of G-protein, which links MOR and DOR to inhibition of Ca 2+ channels and to activation of K + channels (7–12). Inhibition of Ca 2+ channel function will decrease neurotransmitter release from enteric nerve endings, whereas activation of K + changes causes a hyperpolarization of membrane potential and this reduces the probability of action potential firing by the affected neurons. κ-Opioid receptors (KORs) link via G o to inhibition of nerve terminal Ca 2+ channels, also resulting in a decrease in neurotransmitter release (13,14) (see Figure 1). The clinically relevant actions (analgesia, constipation, respiratory depression) of opiate drugs like morphine are mediated predominately by the MOR.

**Cellular and molecular actions of opioid drugs in the ENS**

**Mechanisms responsible for opioid drug-induced changes in motility**—Gut motility is controlled in large part by the myenteric plexus division of the ENS. The myenteric plexus controls contractions and relaxations of the longitudinal and circular muscle layers by releasing excitatory (principally, acetylcholine, and substance P) (15) and inhibitory neuromuscular transmitters (ATP/βNAD, nitric oxide, and vasoactive intestinal peptide) (15–17). Myenteric motor neuronal activity is controlled by inter neurons whose activity coordinates the timing of contraction and relaxation required for production of propulsive motility patterns such as the peristaltic reflex. Interneurons use acetylcholine as the primary excitatory neurotransmitter, but ATP and 5-HT also contribute to fast excitatory interneuronal synaptic transmission (18).

Electrophysiological methods are the principal tools used to investigate the cellular mechanism of opiate action on enteric neurons. Morphine causes membrane hyperpolarization that is associated with a decrease in input resistance (12,14). The reversal potential for the hyperpolarization occurred at the K + equilibrium potential, indicating that the opiate receptor coupled to K + channel activation. MOR, DOR, and KOR also link to inhibition of Ca 2+ channel function (10,11,14). Opioid receptor-mediated inhibition of the Ca 2+ channel function also uses direct coupling of the receptor to the G-protein βγ subunit, which inhibits opening of the Ca 2+ channel (10,11). Inhibition of the nerve terminal Ca 2+
channel function will suppress neurotransmitter release and alter gut motility. Studies of small and large intestinal preparations maintained in vitro have been used to identify the sites of action of opioid drugs on gut motility. Studies on the human colon have shown that morphine acts at MOR and DOR to inhibit inhibitory neuromuscular transmission (19). This will lead to an increase in smooth muscle tone and a decrease in propulsive motility. This is an important mechanism for the constipating effects of morphine and other opioid receptor agonist drugs. In addition, suppression of inhibitory neuromuscular transmission is likely responsible for the cramps caused by opioid drugs.

In addition to motorneurons and interneurons, there is a third type of neuron in the ENS that functions as an intrinsic primary afferent or sensory neuron. These neurons have cell bodies in the myenteric and submucosal ganglia and they are bipolar neurons with an axon projecting into the mucosal villi and a second axon synapsing with interneurons or motorneurons in submucosal or myenteric ganglia. These sensory neurons are the first neurons in reflex pathways activated by mucosal stimulation. These axon terminals in the mucosa express chemical and mechanical sensitive ion channels that respond to chemical and mechanical stimulation of the mucosa. Action potentials propagate along the axon back through the cell bodies and then down the axon that ramifies in the submucosal or myenteric ganglia. These sensory neurons express a Ca\(^{2+}\)-activated K\(^+\) channel that mediates a long-lasting action potential after-hyperpolarization. This after-hyperpolarization limits the firing rate of the neurons. Morphine increases the duration but not the peak amplitude of the after-hyperpolarization, indicating that the opiate receptor is linked to a mechanism that controls the time course of the increase in intracellular Ca\(^{2+}\) following each action potential (14, 20). In the same neurons, morphine shortened the action potential duration by decreasing the Ca\(^{2+}\)-dependent portion of the action potential, and this decrease in Ca\(^{2+}\) entry should shorten, not lengthen, the duration of the after-hyperpolarization.

The studies described above have focused largely on myenteric neurons from the guinea-pig small intestine. As gene knockout mice have become an important research tool, there have been electrophysiological studies of myenteric neurons from the mouse that have revealed additional mechanisms of opiate action (21). These studies used murine myenteric neurons maintained in primary culture along with whole-cell patch clamp methods to study the ionic basis of the inhibitory effects of morphine. These studies revealed that morphine could reduce myenteric neuronal activity by coupling to inhibition of voltage-gated Na\(^+\) channels. This effect would reduce action potential firing and suppress interneuronal signaling and signaling to the muscle layers, although the opioid receptor subtype(s) and signaling mechanism were not identified (21).

**Mechanisms responsible for opioid drug-induced changes in intestinal secretion**—Opioid receptor agonists can inhibit colon water and electrolyte secretion and this effect can contribute to opioid-induced constipation. Secretomotor neurons are localized in the submucosal plexus, and the axons of these neurons project into the mucosal layer where they come into close apposition with epithelial cells (15). The epithelial cells express several types of chloride channels, including CIC2 (22). Submucosal secretomotor neurons release acetylcholine and VIP, which activate muscarinic and VIP receptors on the basolateral surface of the enterocytes (15), which can activate epithelial cell chloride...
channels. Chloride moves from the enterocyte cytoplasm into the gut lumen. H\textsubscript{2}O follows chloride via an osmotic mechanism. Opioid drugs activating MOR and DOR on secretomotor neurons suppress acetylcholine and VIP release, resulting in a decrease in chloride secretion and osmotic water movement (\textsuperscript{8,22}).

\textbf{Cellular and molecular mechanisms of tolerance to the effects of opioid receptor agonists}

Chronic morphine treatment for the relief of pain results in tolerance whereby increased doses are required to maintain response. There are several mechanisms of tolerance to the actions of opioid receptor agonists, and these differ in their time course as tolerance develops. The development of cellular tolerance following activation of G-protein-coupled receptors has been attributed in part to the desensitization and internalization of the activated receptor by the class of proteins termed arrestins (\textsuperscript{23,24}). In particular, \(\beta\)-arrestin-2 has been reported to have an integral role as a scaffolding protein for the internalization of G-protein-coupled receptor through clathrin-coated pits and subsequent intracellular trafficking to the endosome (\textsuperscript{2,25,26}). When an agonist binds to an opiate receptor, it activates the G-protein-dependent signaling mechanisms described above. The activated receptor becomes a substrate for a G-protein receptor kinase, which can phosphorylate specific amino acids found on the intracellular portions of the receptor. Receptor phosphorylation results in acute receptor desensitization that develops within 1 min of receptor activation. The receptor can either be dephosphorylated and reactivated or \(\beta\)-arrestin-2 can be recruited to the phosphorylated receptors, prolonging desensitization by signaling for receptor internalization into the endosome. The receptor can be dephosphorylated in the endosome causing \(\beta\)-arrestin-2 to fall off the receptor, which signals for receptor recycling back to the plasma membrane for another round of opioid agonist receptor activation (\textsuperscript{2,25,26}). This canonical pathway for receptor desensitization does not follow for all opioid agonists. Morphine, for example, produces profound tolerance, yet results in markedly less internalization. Several theories have been proposed to explain these differences. This includes different signaling pathways of MOR phosphorylation by low- and high-efficacy opioid agonists. Morphine-induced analgesic tolerance can be reversed by protein kinase C inhibitors, suggesting that protein kinase C and not G-protein receptor kinase phosphorylation mediates morphine-induced tolerance, whereas the tolerance induced by the high-efficacy opioid agonist DAMGO is mediated via G-protein receptor kinase (\textsuperscript{27}). MOR internalization occurs in myenteric neurons after MOR agonist treatment \textit{in vivo} (\textsuperscript{28}) and \textit{in vitro} (\textsuperscript{29}). This response was highly agonist-dependent with morphine being a very poor activator of the MOR internalization mechanism and this could contribute to the development of opioid-induced bowel dysfunction as described below.

One of the challenges associated with treating chronic pain with opiate receptor agonists is that tolerance develops to the analgesic but not to the constipating effects of agonists. Recent studies suggest that this may be due to differences in receptor desensitization pathways involving \(\beta\)-arrestin-2 in the ENS. Tolerance developed to repeated administration of morphine in the small intestine but not in the colon (see Figure 2) (\textsuperscript{30}). However, tolerance could be induced in the colon of \(\beta\)-arrestin-2 knockout mice (\textsuperscript{31}). In the ileum, prolonged exposure to morphine resulted in decreased \(\beta\)-arrestin-2 expression. This was not observed in the colon, implicating that the decrease in \(\beta\)-arrestin-2 associates with tolerance.
development in the ileum but not in the colon. In contrast, tolerance to the analgesic effects of morphine upon chronic administration is abrogated in the β-arrestin-2 knockout mice (32). When acutely administered, morphine reduces constipation in the β-arrestin-2 knockout mice (33). These seemingly opposite effects of the role of β-arrestin-2 in tolerance development of the central nervous system pain pathways and in the ENS indicate that cellular mechanisms affecting opioid-induced constipation upon prolonged use may also involve differences in receptor signaling mechanisms. Recent studies have identified at least 31 splice variants of the OPRM-1 gene that encodes for the MOR, largely identified in the central nervous system (34). Alternative splicing event occurs in humans and rodents, and points toward the multitude of mechanisms that relate to tolerance development to different opioid agonists. Differences in the carboxy terminal end of the isoforms result in differential cellular signaling and may explain why cross-tolerance to analgesia does not occur among different opioid agonists (35). The presence of differences in MOR mediating the central inhibition of gastrointestinal transit was suggested by earlier pharmacological studies with MOR antagonist naloxonazine (36) where anti-transit effects of intrathecally administered morphine were blocked by the antagonist but not when morphine was delivered at supraspinal levels via intracerebroventricular injections. This was in contrast to the inhibition by naloxanazine of the analgesic effects. More recently, Mori et al. (37) suggested the differential activation of MOR at central and peripheral sites by morphine, oxycodone, and fentanyl. Thus, identifying the specific isoforms in the gastrointestinal tract will be important to establish new receptor targets for treating opioid-induced bowel dysfunction.

A significant development in opioid pharmacology has been the identification of biased agonism. This stems from the realization that a drug’s efficacy to stimulate G-protein activation can be distinguished from its efficacy for β-arrestin-2 recruitment (38). As stated above, in the β-arrestin-2 knockout mice, acutely administered morphine reduces constipation (33), indicating that persistent opiate receptor signaling in the colon via β-arrestin-2 (leading to decreased propulsive motility and colonic secretion) could be an important contributor to the development of opioid-induced constipation. Thus, opioid agonists that do not recruit β-arrestin-2 would result in reduced constipation. TRV130 is a G-protein-biased ligand with less β-arrestin-2 recruitment compared with morphine, with higher potency toward analgesic effects and reduced constipation (39). The potential role of these biased ligands in long-term use is unclear.

**SUMMARY AND CONCLUSIONS**

Morphine and other opioid receptor agonists cause constipation by disrupting neurotransmission in the ENS. This causes a reduction in propulsive motility and colonic chloride secretion. Morphine and other agonists act at MOR, DOR, and KOR to inhibit Ca\(^{2+}\) and Na\(^{+}\) channels and activate K\(^{+}\) channels on enteric neurons. Receptor desensitization is a key component regulating opiate receptor signaling in the nervous system, and β-arrestin binding to activated opiate receptors is a key event in causing receptor internalization and desensitization. There are differences in β-arrestin signaling that are important for tolerance development to the analgesic effects of morphine and other opioid receptor agonists. However, β-arrestin signaling does not link to opioid receptor desensitization and tolerance in the colon. This is likely one cellular/molecular mechanism responsible for opioid-induced constipation.
bowel dysfunction. Development of opioid receptor agonists with biased agonism and identification of receptor isoforms in the gastrointestinal tract are potential areas that require further studies.

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Figure 1.
Signaling pathways activated by opioid receptor agonists in the enteric nervous system. μ- and δ-opioid receptors couple to inhibition of adenylate cyclase via the inhibitory G-protein, Gi. These receptors also couple to activation of K⁺ channels and inhibition of Ca²⁺ channels via a Go-linked pathway. These mechanisms suppress enteric neuronal activity. κ-Opioid receptors link to inhibition of Ca²⁺ channels on enteric nerve terminals. This would inhibit neurotransmitter release. These pathways contribute to the constipating effects caused by opioid receptor agonists. cAMP, cyclic 3’,5’ adenosine monophosphate; PKA, protein kinase A.
Figure 2.
Activation of μ-opioid receptor induces G-protein and β-arrestin-2 signaling pathways. G-protein activation allows for signaling to inhibition of adenylate cyclase and modulation of ion channels. Activation of the β-arrestin-2 pathway, in chronic activation, results in tolerance due to β-arrestin-2-mediated receptor degradation in the ileum, while maintained β-arrestin-2 levels prevent tolerance development in the colon. PKA, protein kinase A.