Insights into the Role of Opioid Receptors in the GI Tract: Experimental Evidence and Therapeutic Relevance

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Contents

Abstract

Opioid drugs are prescribed extensively for pain treatment but when used chronically they induce constipation that can progress to opioid-induced bowel dysfunction. Opioid drugs interact with three classes of opioid receptors: mu opioid receptors (MORs), delta opioid receptors (DOR), and kappa opioid receptors (KORs), but opioid drugs mostly target the MORs. Upon stimulation, opioid receptors couple to inhibitory Gi/Go proteins that activate or inhibit downstream effector proteins. MOR and DOR couple to inhibition of adenylate cyclase and voltage-gated Ca^{2+} channels and to activation of K^+ channels resulting in reduced neuronal activity and neurotransmitter release. KORs

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couple to inhibition of Ca^{2+} channels and neurotransmitter release. In the gastrointestinal tract, opioid receptors are localized to enteric neurons, interstitial cells of Cajal, and immune cells. In humans, MOR, DOR, and KOR link to inhibition of acetylcholine release from enteric interneurons and motor neurons and purine/nitric oxide release from inhibitory motor neurons causing inhibition of propulsive motility patterns. MOR and DOR activation also results in inhibition of submucosal secretomotor neurons reducing active Cl^- secretion and passive water movement into the colonic lumen. Together, these effects on motility and secretion 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 differences in trafficking and downstream signaling in enteric nerves in the colon compared to 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 drug or other treatment strategies of opioid-induced bowel dysfunction.

Keywords
Constipation • Drug tolerance • Enteric nervous system • Opiates

1 Introduction

Opioid receptor agonists are very effective in treating pain and they have powerful effects on gastrointestinal functions. Opioid receptor agonists produce their effects by interacting with opioid receptors, the MOR (the predominant receptor), DOR, and KOR, that belong to the family of G protein-coupled receptors (GPCRs) (Alex et al. 2002; Williams et al. 2013). Some opioid receptor agonists can also be used to treat gut motor and secretory disorders, especially diarrhea. However, chronic administration of opioid agonists causes constipation and in severe cases these drugs can cause the narcotic bowel syndrome (Grunkemeier et al. 2007). With the increased use of prescription opioid receptor agonists for pain treatment, there has been a parallel increase in the number of peripherally restricted opioid receptor antagonists that are used to reverse the gastrointestinal effects of opioid receptor agonists. At the same time peripherally restricted opioid receptor antagonists preserve the central nervous system-mediated analgesic effects of the agonists. Finally, there are peripherally restricted opioid receptor agonist drugs that are used to treat diarrhea but have no abuse potential. This chapter reviews the physiology and pharmacology of opioid receptors in the gut and the mechanisms by which opioid receptor agonists and antagonists alter gut function.

2 Localization of Opioid Receptors in the Gastrointestinal Tract

MOR, DOR, and KOR are expressed by myenteric and submucosal plexus neurons in the enteric nervous system (ENS) (Bagnol et al. 1997; Poonyachoti et al. 2002; Ho et al. 2003; Sternini et al. 2004; Gray et al. 2006; Poole et al. 2011; Lay et al. 2016), with some species differences. In the rat, MOR neurons are more abundant in the submucosal than myenteric plexus, whereas KORs are more numerous in the myenteric plexus, with both MOR and KOR myenteric neurons being more abundant in the stomach and proximal colon compared to other regions of the GI tract (Bagnol et al. 1997). MOR and KOR are not co-expressed in the same rat enteric neurons (Gray et al. 2006), whereas MOR and DOR extensively colocalize in rat and mouse enteric neurons (Gray et al. 2006; Poole et al. 2011). All three opioid receptors are expressed by interstitial cells of Cajal (ICCs) where MOR colocalizes with DOR or KOR in the rat GI tract (Bagnol et al. 1997; Gray et al. 2006). In the mouse GI tract, MOR and DOR but not KOR are expressed by ICC (Kim et al. 2016). In the guinea pig, MORs are localized to interneurons controlling the peristaltic reflex and to submucosal secretomotor neurons (Ho et al. 2003; Lay et al. 2016). In the guinea pig stomach, MORs are located in nitrergic inhibitory motor neurons and cholinergic secretomotor neurons (Lay et al. 2016). In the guinea pig ileal myenteric plexus, MORs are localized to cholinergic, excitatory motor neurons and vasoactive intestinal peptide (VIP) expressing inhibitory motor neurons, and excitatory, cholinergic/VIPergic interneurons (Ho et al. 2003; Lay et al. 2016). The density of MOR nitrergic neurons is much higher than that of MOR cholinergic neurons in the myenteric plexus of both the proximal (Anselmi and Sternini unpublished observations), and distal colon (Anselmi and Sternini unpublished observations; Lay et al. 2016). In the submucosal plexus, MORs are confined to VIP non-cholinergic secretomotor neurons of the ileum and distal colon (Lay et al. 2016). MORs are not expressed by intrinsic primary afferent neurons (IPANs) at least in the guinea pig intestine (Ho et al. 2003; Lay et al. 2016). Finally, MOR nerve fibers are dense in the muscle layers often in close association with ICCs (Ho et al. 2003). Both DOR and KOR have been reported in guinea pig enteric neurons with DOR being localized to myenteric and submucosal neurons and to varicose nerve fibers surrounding nerve cell bodies and the mucosal glands. KOR is confined to the myenteric plexus, where it is localized to neurons and nerve fibers supplying the muscle layers (Sternini et al. 2004). In the mouse gut, DOR-expressing neurons are most abundant in the small intestine and include secretomotor and vasomotor neurons of the submucosal plexus and excitatory and inhibitory myenteric motor neurons in the small intestine, but DOR are expressed mostly by inhibitory motor neurons in the colon myenteric plexus (Poole et al. 2011). Finally, in the human gut, MOR is localized to neuronal cell bodies and nerve fibers in both submucosal and myenteric ganglia of the small and large intestine (Sternini et al. 2004). The overall distribution of opioid receptors is consistent with their role in modulating gastrointestinal motility and secretion (see below).

Opioid receptors, particularly MOR, are expressed by immune cells, supporting a role of the opioid system in regulating intestinal inflammation and intestinal

ischemia (Stefano et al. 1996; Madden et al. 1998; Philippe et al. 2003, 2006; Sternini et al. 2004; Saccani et al. 2012; Anselmi et al. 2015). Opioid receptor activation on immune cells can have indirect actions on enteric nervous system function by suppressing synthesis or release of inflammatory mediators (Hughes et al. 2016) (Table 1).

3 Actions of Opioid Drugs on Myenteric Neurons and Gut Motility

Opioid receptors are G protein-coupled receptors that activate multiple effector molecules. MOR and DOR expressed by myenteric nerve cell bodies couple to the G_i G protein to cause inhibition of adenylate cyclase, reduced cyclic 3′,5′ adenosine monophosphate (cAMP) and reduced levels of protein kinase A (PKA) activation (Liu and Anand 2001; Christie 2008). 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 activation of K^+ channels via a membrane-delimited mechanism (Morita and North 1982; Mihara and North 1986; North et al. 1987; Surprenant et al. 1990; Tatsumi et al. 1990; Shen and Surprenant 1991). Inhibition of Ca^{2+} channels decreases neurotransmitter release from enteric nerves while activation of K^+ channels causes membrane potential hyperpolarization and inhibition of action potential firing. KOR links via G_o to inhibition of nerve terminal Ca^{2+} channels causing decreased neurotransmitter release (Cherubini et al. 1985; Cherubini and North 1985).

Myenteric neurons control gut motility by releasing acetylcholine and substance P to cause muscle contraction (Brookes 2001) and ATP/βNAD, nitric oxide, and VIP to cause muscle relaxation (Jin et al. 1996; Brookes 2001; Hwang et al. 2011). Motor neurons are controlled by interneurons which coordinate the timing of contraction and relaxation required for propulsive motility patterns such as peristalsis. Interneurons use acetylcholine as the primary excitatory neurotransmitter but ATP and 5-HT also contribute to myenteric fast excitatory synaptic transmission (Galligan et al. 2000).

Studies in human colon have shown that morphine acts at MOR and DOR to inhibit inhibitory neuromuscular transmission causing an increase in muscle tone and a decrease in propulsive motility (Bauer et al. 1991). This is an important mechanism for the constipating effects of opioid receptor agonists. In addition, suppression of inhibitory neuromuscular transmission is likely responsible for the abdominal cramps caused by opioid receptor agonists. DOR also mediated inhibition of excitatory cholinergic and non-cholinergic neuromuscular transmission in the human distal colon (Chamouard et al. 1994). Studies done by the same group also showed that KORs mediate inhibition of excitatory cholinergic and non-cholinergic and inhibitory neuromuscular transmission in the human colon (Chamouard et al. 1993).

Studies done in the mouse myenteric plexus have revealed additional mechanisms of opiate action on enteric neurons (Smith et al. 2012). Whole-cell patch-clamp studies using mouse myenteric neurons maintained in primary culture showed that morphine could reduce action potential firing by coupling to inhibition of voltage-gated Na^+ channels. This effect would suppress interneuronal and neuromuscular transmission

Table 1 Summary of opioid receptor localization and function in the gut

Opioid receptor	Cell type expression	Molecular targets	Functional consequence
MOR	Myenteric inhibitory motor neurons (guinea pig stomach and small intestine)	K^+ channel activation	Inhibit action potential firing and neurotransmitter release
		Ca^{2+} channel inhibition	Decreased propulsive motility
	Myenteric excitatory motor neurons, inhibitory motor neurons, and interneurons (guinea pig small intestine)	K^+ channel activation	Inhibit action potential firing and neurotransmitter release
		Ca^{2+} channel inhibition	Decreased propulsive motility
	Myenteric interneurons (guinea pig)	K^+ channel activation	Inhibit action potential firing and neurotransmitter release
		Ca^{2+} channel inhibition	Decreased propulsive motility
	Submucosal secretomotor neurons (guinea pig ileum and distal colon)	K^+ channel activation	Decreased water and electrolyte secretion
		Ca^{2+} channel inhibition	
	Myenteric neurons (mouse); primary culture	Na^+ channel inhibition	Inhibit action potential firing
			Decreased propulsive motility
	Interstitial cells of Cajal (ICC) (rat, mouse)	K^+ ATP channel activation (mouse)	Inhibit pacemaker potentials
	Myenteric and submucosal neurons (human small and large intestine)	Not determined	Decreased water and electrolyte secretion
	Inhibitory motor neurons (human)	Not determined	Decreased muscle relaxation
	Rat myenteric and submucosal neurons	Not determined	Not determined
DOR	Submucosal secretomotor neurons (guinea pig ileum)	K^+ channel activation	Decreased water and electrolyte secretion
		Ca^{2+} channel inhibition	
	Submucosal secretomotor and vasomotor neurons (mouse small intestine)	Not determined	Not determined
	Excitatory and inhibitory motor neurons (mouse small intestine)	Not determined	Not determined
	Inhibitory motor neurons (mouse colon)	Not determined	Not determined

(continued)

Table 1 (continued)

Opioid receptor	Cell type expression	Molecular targets	Functional consequence
	Interstitial cells of Cajal (ICC) (rat, mouse)	K^+ ATP channel activation (mouse)	Inhibit pacemaker potentials
	Excitatory motor neurons (human colon)	Not determined	Decreased neurogenic contraction
	Inhibitory motor neurons (human)	Not determined	Decreased propulsive motility
KOR	Myenteric motor neurons (guinea pig)	Ca^{2+} channel inhibition	Inhibit neurotransmitter release
			Decreased neurogenic contraction and relaxation
	Excitatory and inhibitory motor neurons (human colon)	Not determined	Decreased propulsive motility
	Interstitial cells of Cajal (ICC) (rat)	Not determined	Functional data unavailable

(Smith et al. 2012). Myenteric neurons in the guinea pig and mouse small intestine express nicotinic receptors composed of α3 and β4 subunits and these receptors mediate most fast excitatory postsynaptic potentials in the myenteric plexus (Zhou et al. 2002; Gade et al. 2016). Studies of mouse small intestinal myenteric neurons maintained in primary culture showed that nicotine-induced inward currents were larger in neurons exposed to morphine for 16–24 h compared to neurons exposed to morphine for 1 h. An α3β4 nicotinic receptor agonist increased fecal pellet output in mice treated chronically but not acutely with morphine. These data suggest that tolerance to the inhibitory effects of morphine on gut motility may be due in part to upregulation of α3β4 nicotinic receptors on small intestinal myenteric neurons (Gade et al. 2016).

Recent studies have shown that activation of MOR and DOR but not KOR inhibits pacemaker potentials in mouse intestinal ICC maintained in primary culture. This effect was blocked by glibenclamide, a K^+ ATP channel inhibitor, and by guanylate cyclase and protein kinase G (PKG) inhibitors. These data indicate that MOR and DOR agonists activate K^+ ATP channels via a cGMP/PKG-dependent pathway to inhibit ICC function at least in the mouse intestine (Kim et al. 2016). Disruption of pacemaker potentials would disrupt propulsive motility patterns and this would contribute to the constipating effects of opioid receptor agonists.

4 Actions of Opioid Drugs on Submucosal Neurons and Intestinal Secretion

Opioid receptor agonists inhibit colonic water and electrolyte secretion which contributes to opioid-induced constipation. Water and electrolyte (Cl^-) secretion by enterocytes is stimulated by submucosal secretomotor neurons that release Ach and VIP from nerve endings in close apposition to the enterocytes (Brookes 2001).

Enterocytes express muscarinic cholinergic receptors and VPAC1 and VPAC2 receptors for VIP (Banks et al. 2005). Intestinal water secretion occurs in response to activation of enterocyte Cl^- channels including the cystic fibrosis transport regulator (CFTR) and ClC2 channels (Fei et al. 2010; Kopic et al. 2010). Opioid agonists acting at MOR and DOR on secretomotor neurons suppress Ach and VIP release resulting in a decrease in Cl^- secretion and osmotic water movement (North et al. 1987; Fei et al. 2010; Kopic et al. 2010).

5 Opioid Receptor Trafficking, Signaling Cascades, and Tolerance Development

MOR agonists activate GPCR-dependent pathways that regulate ion channels and adenylyl cyclase, or G protein-independent pathways that include scaffolding molecules and kinases (ERK and JNK). Activation of multiple signaling pathways may reflect agonist selectivity for GPCRs or agonist-selective MOR signaling (Williams et al. 2013). Opioid receptor agonists initiate a cascade of events including phosphorylation of the opioid receptor by G protein receptor kinases (GRKs) that promote receptor interaction with β-arrestins (β-arrestins 1 and 2). Activation of β-arrestin-2 in the ENS uncouples MOR from G proteins, causing internalization of the MOR through clathrin-coated pits and subsequent intracellular trafficking to the endosome (Sternini 2001; Claing et al. 2002; Williams et al. 2013). The receptor is dephosphorylated in the endosome causing β-arrestin-2 to fall off the receptor which is then recycled back to the plasma membrane (Sternini 2001; Claing et al. 2002; Williams et al. 2013). Phosphorylation, endocytosis, intracellular sorting, and recycling are important regulatory processes that mediate desensitization, downregulation, and resensitization, events that modulate cellular responsiveness. However, there are differences in the trafficking and recycling pattern depending on the neuron, agonist, and duration of stimulation. In enteric neurons, in vitro and in vivo, MORs undergo rapid concentration-dependent and ligand-selective internalization that persists for as short as 2 h (Lay et al. 2016) or can last 4–6 h (Minnis et al. 2003). Internalized MORs can recycle back to the cell surface, a process that does not require new receptor synthesis. When recycled back to the membrane, MOR can internalize again with little or no loss of total receptor numbers (Minnis et al. 2003). MOR internalization is induced by endogenous opioids, such as enkephalins and endomorphins, by the MOR agonist DAMGO (D-Ala2,N-Me-Phe4,Gly-ol5 enkephalin) that does not cross the blood-brain barrier, by most opiates, including etorphine and fentanyl (Sternini et al. 1996; Minnis et al. 2003) and by loperamide, a peripherally acting MOR agonist (Lay et al. 2016). By contrast, morphine does not induce receptor internalization under the same conditions, even at concentrations that exceed those inducing maximal inhibition of neurogenic stimulation-evoked smooth muscle contraction or inhibition of cAMP formation. The resistance of morphine-activated MORs to internalization has led to the proposal that internalization might protect against tolerance, since morphine has higher propensity to induce tolerance than opiates which induce efficient MOR internalization (e.g., etorphine or fentanyl) (Martini and Whistler 2007). However, this idea has been challenged by the

observation that morphine acquires the ability to induce pronounced MOR internalization in enteric neurons chronically treated with morphine in vivo (guinea pig) and in vitro (rat) (Patierno et al. 2011; Duraffourd et al. 2014). These differences in MOR internalization in response to morphine might be due to differences in the intracellular levels of proteins involved with receptor trafficking. Indeed, the internalization of morphine-activated MORs in neurons chronically exposed to morphine is accompanied by increased dynamin expression and translocation from the intracellular pool to the membrane. This response is prevented by treatment with a dynamin inhibitor or neuronal transfection with a mutant dynamin, in the absence of a change in β-arrestin levels (Duraffourd et al. 2014). By contrast, in enteric neurons chronically exposed to fentanyl, morphine-activated MORs do not internalize and dynamin localization and levels are unchanged (Anselmi et al. 2013). DAMGO, a selective MOR agonist with high internalizing efficiency, retains its ability to induce MOR internalization in neurons chronically stimulated with either morphine or fentanyl. This result indicates that chronic activation of MOR does not impair receptor trafficking in enteric neurons (Patierno et al. 2011; Anselmi et al. 2013). Different ligands might also affect the recycling pathways as suggested by the report that internalized MORs remain in the cytoplasm for at least 2 h following stimulation with loperamide while DAMGO- and morphiceptin- (MOR agonist) activated receptors recycle back to the membrane within 2 h (Lay et al. 2016). Others have shown that agonist-stimulated MOR can remain internalized up to 6 h before recycling back to the membrane (Minnis et al. 2003), Interestingly, MOR is more abundant in the cytoplasm of unstimulated enteric neurons in the colon compared to the small intestine (Anselmi and Sternini unpublished observations). This finding, together with the different proportion of MOR excitatory and inhibitory enteric neurons in the small and large intestine, with higher ratio of excitatory MOR neurons in the ileum and higher percentage of MOR inhibitory neurons in the colon, could provide some explanation why the ileum but not the colon develops tolerance following chronic treatment with morphine (Ross et al. 2008) (see below).

DORs also undergo agonist-stimulated internalization in enteric neurons. Internalization occurs in the soma and neurites and is blocked selectively by a DOR antagonist and is dynamin dependent (Poole et al. 2011). However, unlike MOR, DORs do not recycle to the cell surface and are degraded in lysosomes. Replenishment of DORs at the cell membrane occurs 6–16 h later and requires synthesis of new receptors. The sustained receptor downregulation might play a role in long-lasting tolerance to DOR agonists (Poole et al. 2011). Furthermore, since MOR and DOR colocalize in enteric neurons, we can speculate that heterodimerization might play a role in regulating the neuronal response to chronic use of opioid drugs, since there is evidence that opioid receptor dimerization or heterodimerization modulates receptor function (Jordan and Devi 1999; Gomes et al. 2004).

As described above, receptor phosphorylation results in acute receptor desensitization that develops within 1 min of receptor activation. Phosphorylated receptors then undergo internalization and intracellular trafficking that induce late desensitization, which is followed by recycling so the receptors can be reactivated or degradation into lysosomes, thus resulting in downregulation (Martini and Whistler 2007; Williams et al. 2013). This canonical pathway for receptor desensitization

does not follow for all opioid agonists as exemplified by morphine, which produces profound tolerance yet results in little or no 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 (PKC) inhibitors suggesting that PKC and not GRK phosphorylation mediates morphine-induced tolerance, while the high-efficacy opioid agonist DAMGO-induced tolerance is mediated via GRK (Bailey et al. 2006). MOR agonists can be distinguished by their internalization profiles and downstream effectors, which reflects functional selectivity at GPCR or ligand-directed signaling. For instance, morphine, unlike DAMGO or fentanyl, does not induce phosphorylation of the downstream signaling mitogen-activated protein kinase/extracellular signal-regulated kinases 1 and 2 (MAPK/ERK) in normal enteric neurons. By contrast, both internalizing (e.g., morphine and derivatives) and non-internalizing opioids (e.g., DAMGO, fentanyl) activate MAPK/ERK in enteric neurons chronically treated with morphine (Duraffourd et al. 2014), further emphasizing ligand and cell type differences in MOR signaling. Together, these findings support the concept that different receptor and signaling mechanisms contribute to the regulation of opioid drug actions in the gut.

6 Tolerance Mechanisms and Opioid-Induced Constipation

Tolerance develops quickly to the analgesic but not constipating effects of opioid receptor agonists. In addition, tolerance develops to the anti-transit effects of morphine in the small intestine but not in the colon (Ross et al. 2008; Galligan and Akbarali 2014). The mechanism responsible for this effect was addressed in experiments measuring contractions caused by repeated morphine applications to in vitro circular muscle/myenteric plexus rings from wild-type mouse small intestine and colon (Kang et al. 2012). Morphine-induced contraction of small intestinal circular muscle, but not colon circular muscle, declined in amplitude with repeated morphine applications suggesting that morphine tolerance developed in the small intestine but not colon. However, when the same experiment was repeated in tissues from β-arrestin-2 knockout mice, morphine tolerance developed in small intestinal *and* colon circular muscle rings (Kang et al. 2012). Similar data were obtained in guinea pig ileum and colon preparations in vitro where tolerance to the inhibitory effects of morphine on electrically evoked circular muscle contractions developed in ileal but not colon tissues (Kang et al. 2012). It has also been found that intestinal levels of β-arrestin do not change following chronic (4–7 days) morphine treatment in vivo in guinea pigs or in vitro in rat ileum (Patierno et al. 2011; Kang et al. 2012; Duraffourd et al. 2014) suggesting the involvement of additional mechanisms that do not depend on β-arrestin-2 levels. Chronic morphine treatment triggers changes in proteins involved in MOR trafficking such as dynamin upregulation and translocation, and downstream signaling including ERK phosphorylation-dependent activation of the transcription factor, CREB. Furthermore, blockade of this signaling pathway prevents the development of gastrointestinal motility impairment induced by chronic morphine treatment (Duraffourd et al. 2014). Thus chronic morphine treatment alters

MOR downstream signaling in enteric neurons leading to opioid-induced constipation. There are ~31 splice variants of the OPRM-1 gene that encodes for the MOR (Pan 2005). Alternative splicing occurs in humans and rodents suggesting that multiple mechanisms can contribute to tolerance to different opioid receptor agonists. Differences in the carboxy-terminal (a target for GRK phosphorylation) in different receptor isoforms may activate different intracellular signaling pathways that can explain why cross-tolerance to analgesia does not occur among different opioid agonists (Pasternak 2001). The presence of differences in MOR mediating the central inhibition of GI transit was suggested by earlier pharmacological studies with MOR antagonist naloxonazine (Heyman et al. 1988) where anti-transit effects of intrathecally administered morphine were blocked by the antagonist but not when morphine was delivered at supra-spinal levels via intracerebroventricular injections. This was in contrast to the inhibition by naloxonazine of the analgesic effects. More recently, Mori et al. (Mori et al. 2013) 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 receptor pharmacology has been the identification of biased agonism where drug-induced stimulation of G protein signaling differs from its efficacy for β-arrestin-2 signaling (Violin and Lefkowitz 2007) or its bias towards the Gi/o proteins (Manglik et al. 2016). As stated above, persistent opiate receptor signaling in the colon via β-arrestin-2 contributes to opioid-induced constipation. Likewise, prolonged MOR signaling and MAPK/ERK activation induced by long-term opioid treatment in the ileum together with the induction of CREB phosphorylation might also contribute to the development of opioid-induced constipation (Duraffourd et al. 2014). Opioid agonists with reduced efficiency in recruiting β-arrestin-2 have strong analgesic properties with reduced side effects such as respiratory depression and constipation (Raehal et al. 2011). TRV130 is a G protein-biased ligand that causes less β-arrestin-2 recruitment than morphine and with higher potency towards analgesic effects and reduced constipation (DeWire et al. 2013). Similarly, a new drug, PZM21, has been discovered utilizing structure-based computational screening methodology, which has strong bias activity for Gi/o signaling and is an effective analgesic with reduced constipation and abuse liability (Manglik et al. 2016). The potential role of biased ligands in long-term use is under investigation.

7 Drugs Acting at Opioid Receptors in the Gastrointestinal Tract

Loperamide (Imodium) Loperamide is an MOR agonist used to treat diarrhea with limited abuse liability. Loperamide is used to treat occasional episodes of diarrhea (traveler's diarrhea) but it is also used to treat some IBS patients with diarrhea as their predominant symptom. Loperamide is a substrate for P-glycoprotein which is a widely expressed transporter protein (Vandenbossche et al. 2010). Loperamide

has limited oral bioavailability due to the activity of P-glycoprotein expressed by mucosal epithelial cells and it has limited blood-brain barrier permeability due to the action of P-glycoprotein expressed by astrocytes and endothelial cells in the cerebral circulation (Davis et al. 2014). Loperamide acts at MOR in the ENS causing decreased propulsive motility and intestinal secretion (Awouters et al. 1983; Ho et al. 2003; Lay et al. 2016).
While loperamide has been used safely for many years, there has been a recent increase in the number of loperamide overdoses and fatalities (Bishop-Freeman et al. 2016). The increase in loperamide overdoses parallels the rise in the use and abuse of prescription opiate pain medications and the related resurgence in heroin addiction (Daniulaityte et al. 2013). At supra-therapeutic blood levels, loperamide can block cardiac HERG K^+ channels leading to potentially fatal arrhythmias (Eggleston et al. 2017; Kang et al. 2016; Vaughn et al. 2016).

Naloxegol (Movantik) Naloxone is a potent and very selective antagonist of opioid receptors, especially MOR. Naloxone is used by first responders to reverse the potentially fatal effects of an opiate overdose. Naloxone readily crosses the blood-brain barrier to block central sites of action of opioid drugs responsible for the lethal effects of an overdose (cardiovascular and respiratory centers). Naloxone also blocks peripheral sites of opiates including the enteric nervous system. Naloxegol is a pegylated modification of naloxone. Naloxegol is a substrate for the blood-brain barrier P-glycoprotein transporter and together with its large molecular weight (652 g/mol) limits naloxegol penetration across the blood-brain barrier (Bui et al. 2016; Leppert and Woron 2016). Naloxegol is approved for treatment of opioid-induced constipation especially in non-cancer pain patients (Chey et al. 2014; Leppert and Woron 2016).

Methylnaltrexone (Relistor) Methylnaltrexone is a naltrexone analog with a quarternary amine group that is positively charged and this limits its blood-brain barrier permeability (Bader et al. 2013; Webster et al. 2015). Therefore, methylnaltrexone can block peripheral MOR without affecting centrally mediated analgesia. Methylnaltrexone is effective in treating opioid-induced constipation in cancer and non-cancer chronic pain patients.

Eluxadoline (Viberzi) GPCRs can form heterodimeric complexes that increase signaling options and pharmacological responses. For example, MOR and DOR form heteromeric complexes throughout the nervous system (Fujita et al. 2015). MOR or DOR ligands can bind individually to the heteromeric receptor complex to activate the dimeric receptor but binding of a DOR antagonist will increase the activity of agonists at the MOR-binding site (Gomes et al. 2004). Eluxadoline has been approved recently for the treatment of diarrhea-predominant IBS (Lacy 2016). Eluxadoline (known as mu/delta in the earlier literature) is a mixed MOR agonist/DOR antagonist (Wade et al. 2012). Preclinical studies showed that eluxadoline had limited systemic bioavailability after oral administration and its actions were restricted to the gut wall. Eluxadoline inhibited propulsive motility in vivo and

intestinal secretion in vitro in mice, but it did not inhibit the visceromotor response to colorectal balloon distention in rats in vivo. These results are consistent with a local gastrointestinal action of eluxadoline. Eluxadoline reduces diarrhea in IBS-diarrhea patients and constipation is rare (Lembo et al. 2016). The beneficial effects of eluxadoline on gut motility may be related to biased signaling due to the mixed MOR agonist/DOR antagonist properties of the drug. Although evidence documenting MOR/DOR dimers in the gut is not available both receptors are expressed in the ENS. Agonist activation of enteric neuronal MOR initiates β-arrestin signaling and ERK phosphorylation which are likely to cause constipation. However, simultaneous ligand binding to an MOR/DOR heterodimer is coupled to G protein signaling pathways not linked to constipation (Wade et al. 2012).

8 Summary and Conclusions

Morphine and other MOR agonists cause constipation by disrupting neurotransmission in the ENS. This causes a reduction in propulsive motility and colonic secretion. Morphine and other agonists act at MOR, DOR, and KOR to inhibit Ca^{2+} and Na^{+} channels and to activate K^{+} channels on enteric neurons. Receptor desensitization is a key component regulating opiate receptor signaling in the nervous system and β-arrestin and dynamin binding to activated opiate receptors causing receptor internalization, intracellular trafficking, and desensitization. There are differences in β-arrestin signaling that are important for tolerance development to the analgesic effects of 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 bowel dysfunction. In addition, other proteins involved in receptor trafficking such as dynamin and GRKs are likely to play an important role in the mechanisms initiating compensatory downstream events that contribute to opioid agonist-induced side effects (Finn and Whistler 2001; Martini and Whistler 2007; Williams et al. 2013). Development of opioid receptor agonists with biased agonism and identification of receptor isoforms as well as a better understanding of downstream signaling pathways in enteric neurons of different regions of the gastrointestinal tract require further study.

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