G Protein-Coupled Receptors are Dynamic Regulators of Digestion and Targets for Digestive Diseases

Short Title: GPCRs and Digestive Disease

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Abbreviations: AKAPs, A-kinase anchoring proteins; ARRB, beta-arrestin; ARRB2, beta-arrestin2; CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor; DOR, delta opioid receptor; DREADDs, Designer Receptors Exclusively Activated by Designer Drugs; ECE1, endothelin converting enzyme 1; ERK, extracellular signal regulated kinase; GABA, gamma-aminobutyric acid; GERD, gastroesophageal reflux disease; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; IBS, irritable bowel syndrome; KOR, kappa-opioid receptor; MGLUR, metabotropic glutamate receptor; MOR, Mu-opioid receptor; MR, muscarinic receptor; MRGPR, Mas-related GPCR; NAM negative allosteric modulator; NK_1R, neurokinin-1 receptor; OR, opioid receptor; PAM, positive allosteric modulator; PAR, protease-activated receptor; PKA, protein kinase A; PKC, protein kinase C; SP, substance P; SST, somatostatin; SSTR, somatostatin receptor; TGR5, Takeda GPCR 5; VFT, Venus Flytrap Domain.

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Abstract

G protein-coupled receptors (GPCRs) are the largest family of transmembrane signaling proteins. Within the gastrointestinal tract, GPCRs expressed by epithelial cells sense contents of the lumen, and GPCRs expressed by epithelial cells, myocytes, neurons, and immune cells participate in communication amongst cells. GPCRs control digestion, mediate digestive diseases, and coordinate repair and growth. GPCRs are the target of over one third of therapeutic drugs, including many drugs used to treat digestive diseases. Recent advances in structural, chemical, and cell biology research have revealed that GPCRs are not static binary switches that operate from the plasma membrane to control a defined set of intracellular signals. Rather, GPCRs are dynamic signaling proteins that adopt distinct conformations and subcellular distributions when associated with different ligands and intracellular effectors. An understanding of the dynamic nature of GPCRs has provided insights into the mechanism of activation and signaling of GPCRs, and has revealed opportunities for drug discovery. We review the allosteric modulation, biased agonism, oligomerization, and compartmentalized signaling of GPCRs that control digestion and digestive diseases. We highlight the implications of these concepts for the development of selective and effective drugs to treat diseases of the gastrointestinal tract.

Introduction

G protein-coupled receptors (GPCRs) are the largest family of transmembrane signaling proteins, with approximately 800 members in the human genome. GPCRs transmit information about the external environment to the interior of the cell, and thereby control most physiological and pathological processes. Approximately half of GPCRs have a sensory function, and mediate olfaction, taste, perception of light, and pheromone signaling. Other GPCRs detect hormones, neurotransmitters, and paracrine factors, and mediate communication among cells. GPCRs are the target of over one third of therapeutic drugs, which illustrates their importance in disease and therapy [1].

The importance, diversity, and complexity of GPCRs are illustrated by their role in digestion and as targets for digestive disease (**Fig. 1**). GPCRs with sensory functions within the digestive tract include receptors of taste buds for sweet, bitter, and savory tastes [2], receptors of enteroendocrine cells for amino acids and proteins [3], and receptors of colonocytes for luminal proteases [4]. GPCRs also sense the products of the microbiome. For instance, secondary bile acids, which are synthesized by bacteria within the colon, activate Takeda GPCR 5 (TGR5) on enterochromaffin cells and enteric neurons to evoke peristalsis [5]. TGR5 expressed by cutaneous sensory nerves has been implicated in cholestatic pruritus [6, 7]. GPCRs of epithelial cells, myocytes, enteric neurons, and immune cells participate in cell-to-cell communication in the digestive system. They include receptors for structurally diverse ligands, including biogenic amines (catecholamines, histamine, serotonin), eicosanoids, amino acid transmitters, purine nucleotides, and neuropeptides and peptide hormones, and proteins. Thus, GPCRs orchestrate digestion (secretion, motility, transport), control disease processes (diseases of motility, secretion, inflammation, pain), and regulate growth and repair. Drugs that activate or inhibit GPCRs are effective therapies for digestive diseases (**Fig. 1**).

Although the endogenous ligands of many GPCRs are known, there remain approximately 100 GPCRs with unidentified natural ligands. Some of these orphan GPCRs have roles in the digestive system. For example, the Mas-related GPCR (MRGPR) family comprises approximately 40 orphan receptors expressed by primary sensory neurons and mast cells [8]. MrgprX2 (human) or MrgprB2 (murine homolog) is expressed by mast cells and mediates antibody-independent responses to basic secretagogues, including drugs and peptides associated with pseudo-allergic reactions [9]. Substance P (SP), a gut neuropeptide, can

activate MrgprX2. Mast cells are in proximity to sensory nerves containing SP and calcitonin gene-related peptide (CGRP) in the intestine [10]. Therefore, it is possible that neuropeptides and MrgprX2 mediate communication between sensory nerves and mast cells. Communication between sensory neurons and mast cells has been implicated in irritable bowel syndrome (IBS) [11].

GPCRs share a conserved structure with seven transmembrane domains, three extracellular and three intracellular loops, and extracellular (N-terminal) and intracellular (C-terminal) tails of varying sizes. GPCRs are grouped into five families based on structural and functional similarities. The rhodopsin family (class A) includes receptors for neurotransmitters, peptides, visual pigments, odorants, tastants, and pheromones. The secretin family (class B) comprises receptors for polypeptide gut hormones, including glucagon, glucagon-like peptides, glucose-dependent insulinotropic polypeptide, secretin, vasoactive intestinal peptide, pituitary adenylate cyclase-activating polypeptide, and growth-hormone-releasing hormone. The glutamate family (class C) includes metabotropic glutamate receptors, a calcium-sensing receptor, and gamma-aminobutyric acid (GABA) $GABA_B$ receptors. Adhesion family GPCRs possess a large extracellular N-terminus that is cleaved during activation. The frizzled family, which includes Frizzled and Smoothened proteins, are activated by lipoglycoproteins of the Wnt family (Frizzled) and Hedgehog family (Smoothened). All GPCR families are represented within the digestive system.

This review highlights how recent advances in structural, chemical, and cellular biology research have provided an understanding of the mechanism of action of GPCRs. The traditional view that GPCRs are simple on and off switches that operate at the surface of cells to control a defined set of intracellular signals has been superseded by the realization that GPCRs are dynamic signaling proteins that can adopt different conformations and subcellular distributions, depending on the mechanisms of their activation [12].

One aspect of the dynamic nature of GPCRs was revealed using X-ray crystallography and cryo-electron microscopy to probe GPCR structures. These approaches provided information about the organization of transmembrane, loop, and tail domains and their association with agonists, antagonists, G proteins, beta-arrestins (ARRBs), and other signaling effectors [13-17]. Limitations of structural studies of GPCRs include a requirement to stabilize receptors and signaling complexes by mutation, fusion to stabilizing proteins, or with single domain antibodies (nanobodies). Moreover, structural studies only

provide snapshots of receptors frozen in time. However, structural analyses have revealed that GPCRs adopt distinct conformations when bound to different agonists, antagonists, and intracellular effector and regulators. Two pharmacological paradigms have emerged from an appreciation of the structural dynamism of GPCRs: allosteric modulation [18] and biased agonism [19]. Structural studies have also provided evidence that certain GPCRs exist as oligomers rather than monomers [20, 21].

A second component of the dynamic nature of GPCRs was discovered using biosensors, biophysical approaches and advanced imaging to study the trafficking and signaling of GPCRs in subcellular microdomains. These studies revealed that GPCRs are motile signaling proteins that, upon activation, can traffic from the cell surface to endosomes by dynamin- and clathrin-mediated endocytosis. GPCRs in endosomes can generate sustained signals in subcellular compartments (i.e., compartmentalized signaling) that control physiological and pathological processes [22-27]. Thus, GPCRs in endosomes, rather than at the plasma membrane, might be a target for therapy [28].

Herein we discuss allosteric modulation, biased agonism, oligomerization, and compartmentalized signaling of GPCRs that control digestion and digestive diseases, and consider the implications of these concepts for the development of drugs to treat gastrointestinal diseases.

Allosteric Modulators of GPCRs: Signaling Rheostats

The Concept of Allosteric Modulation of GPCRs

Allosteric modulators are drugs or endogenous molecules that fine-tune the ability of agonists to activate GPCRs. The challenge of developing drugs that are selective for a particular GPCR subtype illustrates the potential of allosteric modulation for drug discovery. A single endogenous ligand can activate several GPCRs (e.g., acetylcholine activates five muscarinic receptors, $M_{1\text{-}5}$Rs). These GPCR subtypes regulate processes within the digestive system and elsewhere. For example, studies in receptor knockout mice indicate that M_1R and M_3R regulate salivary secretion [29], while M_2R and M_3R control intestinal smooth muscle contraction [30]. M_1R, M_4R, and M_5R function in the central nervous system [31]. Since the binding sites for endogenous ligands (orthosteric sites; right or proper in Greek) are conserved between GPCR subtypes, it is challenging to identify subtype-selective drugs that occupy the same site as the natural ligand. An

alternative approach to attain subtype selectivity is to develop drugs that bind to a different site (allosteric site; other in Greek) [32, 33]. Ligands that interact with allosteric sites can induce changes in GPCR conformation that potentiate (positive allosteric modulators, PAMs) or inhibit (negative allosteric modulators, NAMs) endogenous agonists (**Fig. 2**). Intracellular effectors, including G proteins and ARRBs, are physiological allosteric modulators, since interaction with GPCRs induces changes in conformation that alter agonist affinity [34, 35].

There are advantages to drugs that interact with allosteric rather than orthosteric sites. First, allosteric modulators might provide subtype selectivity, as the allosteric site is likely to be less conserved than the orthosteric site, which evolved to bind the same endogenous transmitter. Second, allosteric ligands modulate the activity of GPCRs that are bound to endogenous ligands, providing an opportunity to fine-tune physiological responses. Finally, as the magnitude of an allosteric effect is limited by cooperativity between orthosteric and allosteric sites, allosteric ligands have a ceiling level beyond which no further modulation occurs, with reduced propensity for overdose and toxicity. These advantages have led to drug discovery efforts focused on the identification of allosteric modulators of GPCRs [18], some of which have progressed to clinical trials [1]. However, there are only two approved allosteric modulators of GPCRs: maraviroc, a chemokine receptor 5 NAM that inhibits HIV entry [36], and cinacalcet, a calcium-sensing receptor PAM used to treat hyperparathyroidism [37]. These drugs were found to be allosteric modulators after regulatory approval.

The Translational and Clinical Impact of Allosteric Modulators for Digestive Diseases

Consideration of the clinical utility of allosteric modulators of GPCRs raises two questions: are allosteric modulators a potential treatment for digestive diseases, and will gastrointestinal-related adverse events prohibit use of PAMs and NAMs for non-gastrointestinal disorders? PAMs and NAMs have been developed for several GPCRs found in the gastrointestinal tract; some have progressed to clinical trials (**Table 1**).

PAMs and NAMs have been identified for $M_{1\text{-}5}R$ [38]. Allosteric targeting of M_1R, M_4R, and M_5R is an attractive treatment for disorders of the central nervous system, including schizophrenia, where subtype-specificity would limit off-target effects on peripheral M_2R and M_3R, which are expressed in the digestive

tract [39]. The M_1R PAM benzyl quinolone carboxylic acid (BQCA) improves cognitive deficits but induces diarrhea in mice [40, 41]. Compounds with differential positive cooperativity across subtypes could improve cognition with a lower risk of gastrointestinal side effects [42]. MK-7622, a M_1R PAM, sensitizes the M_1R to acetylcholine in the nanomolar range with no effect on M_2R, M_3R or M_4R up to 100 μM [43]. MK-7622 improved cognitive testing in preclinical models. Two phase I trials tested MK-7622. MK-7622 produced an increase in sigma band awake electroencephalogram, which indicated alertness. It also reversed the negative cognitive effects induced by scopolamine, a MR antagonist [43]. Based on these results, a phase IIa and IIb, multicenter, randomized, double-blind, placebo-controlled, parallel group trial was undertaken to evaluate the efficacy and safety of MK-7622 as an adjunctive therapy to acetylcholinesterase inhibitors for Alzheimer's disease (ClinicalTrials.gov Identifier: NCT01852110). The trial was stopped since MK-7622 failed to improve cognition. Diarrhea, which is acetylcholine-induced, was the most common adverse event. Given the prominent role of M_2R and M_3R in regulating gastrointestinal smooth muscle, peripherally restricted allosteric modulators that fine-tune the actions of acetylcholine might offer a potential therapy for motility and secretory disturbances and visceral pain of IBS [44].

Opioids and associated Mu, delta, and kappa opioid receptors (MOR, DOR, KOR, respectively) are expressed throughout the gut. In addition to their analgesic properties, which are mediated by ORs expressed by primary sensory neurons and second order spinal neurons, opioids inhibit intestinal motility and electrolyte and fluid secretion by activating ORs on enteric neurons. Orthosteric agonists of MOR are used to treat pain (e.g., morphine, fentanyl) and diarrhea (e.g., loperamide). However, their usefulness is limited by respiratory depression, constipation and addiction. Morphine-induced analgesia is limited by tolerance (i.e., reduced effectiveness with sustained use). PAMs of MOR could provide effective therapy without adverse effects by amplifying the actions of endogenous opioids or by allowing a reduction of the dose of synthetic opioids. BMS-986122 is a MOR PAM that potentiates opioids and morphine [45, 46]. However, since respiratory depression and constipation are mediated by MOR, PAMs would be expected to potentiate these side effects. While MOR is the prominent target of opioid analgesics, DOR also controls intestinal contractility [47]. DOR is a target for diarrhea-predominant IBS-D [48], and enhancement of enkephalinergic signaling attenuates secretory diarrhea [49]. BMS-986187 is a DOR PAM that amplifies the

actions of DOR agonists [50]. By modulating endogenous opioids, DOR PAMs have the potential to inhibit motility without causing constipation. Despite the promise of the MOR PAM (BMS-986122) and the DOR PAM (BMS-986187), the therapeutic potential of these drugs is yet to be assessed and they have not been tested in clinical trials.

Allosteric modulators of gut GPCRs have been described for the treatment of other digestive disorders. Glutamate, a transmitter of visceral and somatic pain, can activate ionotropic receptors (ion channels) and metabotropic GPCRs (MGLUR1-8). MGLUR5, which is expressed by vagal afferent endings of the gastro-esophageal sphincter, regulates sphincter tone, providing a basis for the development of allosteric modulators of MGLUR5 for gastro-esophageal reflux disease (GERD). ADX10059 is a MGLUR5 NAM. A randomized, patient-blind, placebo-controlled trial demonstrated that ADX10059 reduced GERD-related symptoms [51]. Dizziness developed in 75% of participants. ADX10059 was then tested, at a reduced dose, in a double-blind, placebo-controlled, multi-center trial in participants with proton pump inhibitor-responsive GERD. At this reduced dose, ADX10059 increased symptom- and heartburn-free days and reduced regurgitation and sleep disturbance. Mild to moderate dizziness and vertigo were experienced only by 16% and 12% of patients, respectively [52] (ClinicalTrials.gov Identifier: NCT00820079). Testing was stopped because long term administration of ADX10059 in a trial for the prevention of migraine elevated hepatic transaminases (ClinicalTrials.gov Identifier: NCT00820105). Liver enzyme elevation resulted from metabolism of ADX10059 rather than MGLUR5 inhibition; therefore, negative allosteric modulation of MGLUR5 remains a viable approach for GERD.

Biased-Agonism of GPCRs: Shapeshifting Receptors and Pathway-Selective Drugs

The Concept of Biased Agonism of GPCRs

Biased agonism describes the phenomenon whereby the binding of different ligands, including endogenous ligands or drugs, to the same receptor in an identical cellular background results in differential activation of signaling pathways [19] (**Fig. 3**). While this is the definition of ligand biased agonism, other descriptions include differential localization of activated GPCRs (location bias) or differential signaling between various cell types (system bias). Biased agonism provides an avenue for pathway-selective drug

discovery (i.e., the development of drugs that modulate the beneficial pathways rather than those that give rise to adverse effects). Ligand bias can be attributed to different agonists stabilizing distinct conformations of GPCRs that couple to particular signaling effectors. Studies of serotonin receptors bound to the ARRB-biased agonists ergotamine and lysergic acid diethylamide support this concept [53]. However, robust structural evidence for this mechanism of biased agonism is lacking and will require studies of GPCRs in multiple activation states. The realization that GPCRs can be differentially activated within intracellular compartments (see Compartmentalized Signaling) has sparked interest in location bias as a therapeutic avenue [54]. Finally, system bias, which can be attributed to differences in the stoichiometric ratios of signaling effectors between cells, also offers a strategy for the design of effective therapies. However, these endeavors require an understanding of the signaling pathways in functionally relevant cells and of how they may be altered during disease, which, in most cases, is still lacking. Biased agonism of GPCRs has implications for both physiological control and drug discovery.

The mechanisms by which serine and cysteine proteases activate protease-activated receptor-2 (PAR_2) illustrate the relevance of biased agonism of a GPCR that controls gut functions. PAR_2 is expressed throughout the digestive system, where it regulates inflammation, pain, motility, and secretion, and is a therapeutic target for inflammatory and functional disorders [55]. During disease, proteases become activated and trigger PAR_2 by distinct mechanisms [56]. Trypsins, from pancreatic secretions and colonocytes, and mast cell tryptase cleave within the extracellular N-terminus of PAR_2 at the $R^{36}\downarrow S^{37}$ to reveal a new N-terminal tethered ligand domain (S^{37}LIGKV). This domain then binds to extracellular loops of cleaved PAR_2, which couples to $G\alpha_q$, $G\alpha_s$ and ARRBs. PAR_2 internalizes and can continue to signal from endosomes (see Compartmentalized Signaling) [25, 57]. This canonical mechanism, which operates in model cell lines and primary sensory neurons, was once considered to be the only way proteases could activate PAR_2. However, cathepsin-S from macrophages and neutrophil elastase cleave PAR_2 at different sites from trypsin and tryptase and activate PAR_2 by biased mechanisms [58, 59]. Cathepsin-S cleaves at $E^{56}\downarrow T^{57}$ to reveal a distinct tethered ligand (T^{57}VFSVDEFSA), which binds to PAR_2 and induces coupling to $G\alpha_s$ [58]. Elastase cleaves PAR_2 at $S^{67}\downarrow V^{68}$, close to the first transmembrane domain, and activates the receptor by a mechanism that

likely involves a conformational change rather than exposure of a tethered ligand, and induces PAR_2 coupling to $G\alpha_s$ and $G\alpha_{12,13}$ [59]. After cleavage by cathepsin-S and elastase, PAR_2 neither couples to $G\alpha_q$ nor ARRBs and does not internalize. An understanding of these mechanisms provides insights into how these proteases signal PAR_2-dependent pain, including inflammatory pain in the colon [25]. Trypsin evokes hyperexcitability of primary sensory neurons by mechanisms that depend on protein kinase C (PKC) and extracellular signal regulated kinase (ERK), which are down-stream from $G\alpha_q$ [25]. Cathepsin-S and elastase evoke hyperexcitability of neurons by adenylyl cyclase- and protein kinase A- (PKA) mediated pathways, downstream from $G\alpha_s$ [25, 58, 59]. The mechanisms by which proteases of different selectivity can activate PAR_2 represents biased signaling, where the receptor couples to different G proteins depending on the site of cleavage. Other GPCRs that control gut functions may also be activated by biased mechanisms, although this has not been studied. Biased agonism is likely to be pertinent for GPCRs for neuropeptides, which often exist in multiple forms that might interact with receptors in different ways.

In addition to its physiological relevance, biased agonism of GPCRs has implications for drug discovery. A limitation of most agonist drugs is that the same receptor mediates the beneficial and detrimental effects (i.e., on-target side effects). For example, MOR mediates morphine-induced analgesia, but also causes constipation and respiratory depression. If the signaling pathways that are responsible for the beneficial and detrimental actions of agonists are known, and are different, it may be possible to develop drugs that activate only the beneficial signaling events, thereby minimizing on-target side effects. Such drugs would not only be receptor-specific, but also pathway-specific, offering selectivity (**Fig. 3**). Although this concept is attractive, the development of pathway-selective biased agonists is challenging [60]. The signaling pathways that underlie the beneficial and detrimental actions of agonists in vivo are not always known due to the difficulty of studying signaling in primary cells and intact animals.

Despite these challenges, there has been interest in developing pathway-selective biased agonists of opioid receptors that would treat pain without on-target side effects. Interest in this area was sparked by the observation that mice lacking beta-arrestin2 (ARRB2) displayed altered responses to morphine [61, 62]. ARRB2 deletion enhanced and prolonged morphine-induced analgesia, which is attributable to decreased

MOR desensitization. In contrast, ARRB2 deletion attenuated morphine-induced tolerance, respiratory depression and constipation, which suggests that ARRB2 mediates the signaling that underlies these effects [61-63]. Observations with loperamide, a peripherally-restricted MOR agonist, confirmed that ARRB2 mediates opioid-induced constipation [64, 65]. However, ARRB2 plays a role within the digestive tract, where it mediates the development of tolerance to morphine in the colon but not in the ileum [66-68]. The observation that ARRB2 plays distinct roles in regulating MOR signaling that underlies analgesia versus respiratory suppression and constipation prompted efforts to identify biased agonists of MOR that activate G proteins but not ARRBs. Potentially, G protein-biased agonists would induce analgesia without on-target side effects. Several candidates have emerged.

The Translational and Clinical Impact of Biased Agonists for Digestive Diseases

TRV130 (Oliceridine, OLINVO) is a weak G protein-biased agonist of MOR [69]. Consistent with its reduced ability to recruit ARRB2, TRV130 stimulates minimal MOR phosphorylation or internalization, compared to other opioids [70]. TRV130 retains analgesic activity in rodents, with reduced adverse effects of gastrointestinal function and respiration [70]. ClinicalTrials.gov lists ten trials related to TRV130 (**Table 1**). A double-blind, patient-controlled analgesia phase IIb study was designed to investigate the efficacy, safety and tolerability of TRV130 compared to morphine and placebo in patients with moderate to severe pain following abdominoplasty (ClinicalTrials.gov Identifier: NCT02335294). Although the analgesic efficacy of TRV130 was similar to morphine, TRV130 produced less nausea and vomiting [71]. In healthy men, TRV130 produced greater analgesia than morphine, with a smaller reduction in respiratory function and less nausea and vomiting (ClinicalTrials.gov Identifier: NCT02083315) [72]. These clinical trials do not report whether the incidence of constipation following administration of TRV130 is lower relative to morphine. Oliceridine was granted FDA novel drug application status in 2017, but this application was rejected due to safety issues and dosing concerns.

Structure-based drug design has been used to develop G protein-biased agonists of ORs. PZM21 is a G protein-biased MOR agonist derived from structure-based drug design efforts facilitated by the resolution of the crystal structure of all opioid receptor subtypes [73]. Together with PZM-21, multiple G protein-biased MOR agonists have been identified that provide analgesia with fewer on-target side effects

[74]. However, recent studies suggest that TRV130 and PZM21 retain their undesirable side effects with repeated use despite being G protein-biased [75, 76]. Further studies are required to ascertain the therapeutic utility of G protein-biased agonists of MOR.

Biased agonists of DOR have been tested for analgesic efficacy [77]. The attractiveness of DOR agonists for clinical use is their reduced propensity to inhibit gastrointestinal motility and cause constipation, compared to MOR agonists [78, 79]. The DOR agonists SNC80 and ARM390 produce comparable analgesia but show biased effects at the cellular and behavioral level [80]. Whereas SNC80 causes endocytosis of DOR, ARM390 does not. Repeated injection of SNC80 produces analgesic, locomotor and anxiolytic tolerance, along with receptor downregulation. Repeated administration of ARM390 produces analgesic tolerance, but not locomotor or anxiolytic tolerance. Dorsal root ganglia from the mice treated with these agonists demonstrated intact DOR expression, although DOR coupling to calcium channels was lost. ADL5859 and ADL5747 are DOR agonists that, like ARM390, produce biased effects in preclinical studies [81, 82]. ADL5747 and ADL5859 produce antinociception in inflammatory and neuropathic pain models, do not activate locomotion and do not induce DOR internalization. ADL5859 has been tested in clinical trials for analgesic efficacy following molar removal (ClinicalTrials.gov Identifier: NCT009938363), rheumatoid arthritis (ClinicalTrials.gov Identifier: NCT00626275), diabetes-induced peripheral neuropathy (ClinicalTrials.gov Identifier: NCT00603265), and osteoarthritis (ClinicalTrials.gov Identifier: NCT00979953). ADL5859 did not demonstrate analgesic efficacy in these trials. Preclinical testing showed ADL5747 to have higher analgesic potency than ADL5859 in a model of inflammatory pain in rats. However, it failed to show analgesic efficacy for osteoarthritic pain (ClinicalTrials.gov Identifier: NCT00979953) and postherpetic neuralgia (ClinicalTrials.gov Identifier: NCT01058642).

GPCR Oligomerization: It Takes Two to Tango

The Concept of Oligomerization of GPCRs

Although receptor tyrosine kinases and ion channels can assemble into multimeric functional units, the oligomerization of GPCRs is controversial. In light of this controversy, the International Union of Basic and Clinical Pharmacology has developed criteria for the acceptance of GPCR oligomers [83]. Criteria

include: evidence of physical association of GPCRs in native tissues and cells, rather than in transfected cells; evidence of a new or different pharmacological property of the oligomer in native systems; and the observation of functional changes when one of the protomers is deleted in animals. Despite this controversy, the development of drugs that target components of a GPCR oligomer offers the possibility of selectivity and efficacy (**Fig. 4**).

Oligomerization of Class C GPCRs

The strongest evidence for the existence of dimers comes from the class C GPCRs (glutamate, GABA, calcium). Dimerization of some class C GPCRs is necessary for function, where association of two identical or distinct subunits forms a functional receptor. In contrast to other families, the ligand binding site of these GPCRs is not located within the heptahelical domain, but rather within a large extracellular Venus Flytrap Domain (VFT). Class C GPCR dimers are stabilized by a disulfide covalent linkage between the two subunits. Dimerization of these receptors is essential for allosteric coupling between the VFT and the heptahelical domain and thus between sites for ligand binding and G protein activation. Heterodimerization of the $GABA_{B1}$ and $GABA_{B2}$ receptors is required to mask an endoplasmic reticulum retention sequence, allowing translocation of receptors to the plasma membrane [84-86]. Agonist binding to $GABA_{B1}$ allosterically activates $GABA_{B2}$ to initiate intracellular signal transduction. Although this heteromerization was first described in the brain [85], it has also been postulated to occur in the digestive tract [87] and is supported by the colocalization of both subunits in the upper gut [88]. $GABA_A$ and $GABA_B$ receptors are expressed throughout the gut, and can regulate relaxation of the lower esophageal sphincter, gastric and intestinal motility, and colonic pain [89]. $GABA_B$ agonists have been proposed as a treatment for GERD but the incidence of centrally mediated side effects has limited therapeutic applicability [90].

Oligomerization of Class A GPCRs

The dimerization of class A GPCRs, although more controversial than for class C GPCRs, illustrates the dynamism of this receptor family, since the assembly of class A oligomers has been proposed to be ligand-dependent and to modulate GPCR biogenesis and endocytosis [91, 92] (**Fig. 4**). Dimerization of opioid receptors has attracted attention. Studies of purified receptors reconstituted into a phospholipid bilayer indicate that monomeric MOR can bind agonists and antagonists and is the minimal functional unit

necessary for G protein activation [93]. However, structural and functional observations suggest that opioid receptors can dimerize. Antagonist-bound MOR crystalized as a symmetrical dimer with the interfaces within transmembrane helices 5 and 6 [20], although these interfaces were not observed in the agonist-bound structure [94]. MOR homodimers have been detected in both heterologous expression systems and in vivo [95].

MOR may dimerize with DOR, since in recombinant systems a MOR-DOR heterodimer displays binding and functional properties that can be observed in native membranes of wild-type but not in knock-out mice [96]. However, these data have been debated. In transgenic mice expressing DOR fused to green fluorescent protein (DOR-GFP), there is little overlap between DOR-GFP and immunoreactive MOR in primary sensory and spinal neurons [97], although DOR-GFP and MOR-mCherry are coexpressed in limited neuronal populations [98]. Within pain pathways, DOR-MOR co-expression is limited to excitatory interneurons and projection neurons in the dorsal horn of the spinal cord, and to neurons in parabrachial, amygdalar, and cortical regions of the brain [99]. Within these neurons, DOR and MOR traffic and function independently. Despite this controversy, the MOR-DOR heterodimer has been suggested as a therapeutic target that could provide analgesia with decreased tolerance [100, 101]. Bifunctional ligands, comprising a MOR agonist and a DOR antagonist, have been generated with the rationale that DOR antagonists may enhance MOR responses.

Although functional coexpression of MOR and DOR by the same neuron was first demonstrated using electrophysiological recordings from enteric neurons [102], the definitive demonstration of MOR-DOR heteromers in enteric neurons is lacking. DOR-GFP is coexpressed in a subpopulation of myenteric neurons with immunoreactive MOR [103]. However, whether they form heteromers or functionally interact through other mechanisms has not been determined. Electrophysiological and molecular studies show that MOR and DOR are coexpressed by afferent neurons innervating the mouse colon, where receptors may suppress neuronal excitability during inflammation [104].

The Translational and Clinical Impact of GPCR Oligomers for Digestive Diseases

The utility of bivalent drugs that recognize both components of a GPCR dimer is illustrated by finding that a molecule with MOR agonist and DOR antagonist activity (Eluxadoline) acts through the MOR-DOR heteromer [105] (**Table 1**) (**Fig. 4**). Eluxadoline relieves abdominal pain in patients with IBS-D

(ClinicalTrials.gov Identifier: NCT01553747; NCT01553591) [48, 106]. Despite the MOR activity, the drug showed no evidence of abuse potential in phase II and III clinical studies [107]. A clinical trial is open to test whether Eluxadoline is effective for the management of IBS-D in patients with bile acid malabsorption (ClinicalTrials.gov Identifier: NCT03441581). Eluxadoline will be tested for the management of diarrhea-associated fecal incontinence (ClinicalTrials.gov Identifier: NCT03489265).

Compartmentalized Signaling: Adding Texture to GPCR Responses

The Concept of Compartmentalized Signaling of GPCRs

While alterations in the conformation of GPCRs might account for allosteric modulation and biased agonism, and could explain the altered functions of GPCR oligomers, GPCRs also undergo positional changes during their activation-deactivation cycle, exemplified by agonist-induced endocytosis. Agonist-induced endocytosis in vivo has been demonstrated for the NK_1R and DOR, due to the availability of selective NK_1R antibodies and transgenic mice expressing DOR-GFP. Physiological stimuli evoke NK_1R endocytosis in endothelial cells of post-capillary venules at sites of neurogenic inflammation [108], in enteric neurons during inflammation [109], and in second order spinal neurons after painful stimuli [24, 110, 111]. Exogenous and endogenously-released opioids induce endocytosis of DOR in myenteric neurons [47, 103]. These studies led to the appreciation that GPCRs can signal from endosomes as well as the plasma membrane, with implications for physiological control and drug discovery [23, 26, 28]. GPCRs in endosomes can generate sustained signals in subcellular compartments (i.e., compartmentalized signaling) that contribute to important pathophysiological processes, and endosomal GPCRs could be an important target for therapy.

Control of Plasma Membrane Signaling of GPCRs

Plasma membrane signaling is regulated by ligand degradation and reuptake and by receptor desensitization and endocytosis, and is often transient (**Fig. 5**). Cell surface peptidases degrade neuropeptides and terminate their biological effects. Neprilysin degrades and inactivates SP and bradykinin and attenuates their proinflammatory actions [112-114]. Neprilysin deletion causes NK_1R-dependent plasma extravasation in the digestive tract [115], and exacerbates inflammation of the intestine due to impaired

degradation of SP [114]. Enkephalin-degrading enzymes regulate activation of opioid receptors, and inhibitors of these enzymes suppress diarrhea by enhancing the anti-secretory actions of endogenous opioids [49].

GPCR desensitization also regulates signaling at the plasma membrane. ARRBs uncouple GPCRs from G proteins and couple GPCRs to the clathrin-mediated endocytic machinery [116]. Desensitization of MOR and analgesic tolerance to opioids are associated with a reduction of MOR at the plasma membrane [117]. However, tolerance to morphine develops for pain and for motility of the upper gut but not the colon, leading to constipation with escalating doses of opioids that are required to control pain [68]. Differential functions of ARRBs may account for these differences in tolerance.

Intracellular Signaling of GPCRs

Although endosomes were considered to be a conduit for receptor trafficking to recycling or degradatory pathways, endosomes are now considered to be a major site of continued signaling by GPCRs [22-27, 118-121]. GPCRs in endosomes can assemble signaling complexes (signalosomes) in subcellular compartments. The spatial and temporal characteristics of these signals can provide a mechanism underlying specific cellular responses (**Fig. 5**).

The idea of compartmentalized signaling, while initially proposed for cAMP [122], was first demonstrated for calcium signaling due to the availability of fluorescent indicators that allowed observations of calcium sparks, puffs and blinks within living cells [123]. The use of genetically encoded Förster Resonance Energy Transfer biosensors that are targeted to particular subcellular domains has revealed that most signals are compartmentalized [124]. Signal compartmentalization can be achieved by the formation of signaling microdomains, such as those described for receptors that stimulate the formation of cAMP. Here, local second-messenger concentrations are controlled by the proximity of adenylyl cyclase (generates cAMP), phosphodiesterases (degrade cAMP) and cAMP-activated PKA [125]. Scaffolding proteins that lack enzymatic activity but participate in the organization of signaling effectors can mediate signal compartmentalization. A-kinase anchoring proteins (AKAPs) are recognized for their roles in the formation of multi-protein complexes that modulate spatial and temporal cAMP signaling [125]. ARRBs serve as molecular scaffolds that recruit GPCRs, including PAR_2 and NK_1R, and components of the mitogen-activated protein kinase cascade to endosomes for the activation of ERK in subcellular compartments [57, 126].

Although most descriptions of compartmentalized GPCR signaling in physiological settings have been focused on the heart and brain, signal compartmentalization in the gastrointestinal tract has been reported for cAMP [127].

Control of the Endosomal Signaling of GPCRs

The trafficking of GPCRs through the endosomal system, which depends in part on the stability of agonist-GPCR-ARRB complexes, governs the speed of receptor recycling and resensitization and the duration of endosomal signals. Initially, GPCRs that exhibited sustained interactions with ARRBs were designated Class B GPCRs (e.g., NK_1R, PAR_2) [128, 129] and those that exhibited low affinity and transient interactions with ARRBs were termed Class A GPCRs (e.g., NK_3R, MOR) [130]. While this initial classification has been linked to the dynamics of receptor internalization and recycling, it has become apparent that not all GPCRs fall in these two categories. Despite this, the differential affinity for ARRBs can affect signaling of receptors that are coexpressed in enteric neurons, where the activated NK_1R sequesters ARRBs and thereby inhibits ARRB-dependent desensitization and endocytosis of the NK_3R [130]. This process may provide a mechanism for sustained signaling by tachykinins through the NK_3R even after the NK_1R is desensitized and internalized.

In the case of neuropeptide receptors, degradation of ligands by endosomal peptidases also determines stability of agonist-GPCR-ARRB complexes and controls GPCR trafficking and signaling. Endothelin-converting enzyme 1 (ECE1) is a transmembrane peptidase found in early endosomes of many cells, including enteric neurons and endothelial cells [131-134]. By degrading SP and CGRP in acidic endosomes, ECE1 destabilizes the agonist-GPCR-ARRB complex, which terminates endosomal signaling and promotes receptor recycling and resensitization. This mechanism controls the proinflammatory and neurotoxic actions of SP and the NK_1R [135]. The susceptibility of endogenous peptides and peptidic drugs to degradation by endosomal ECE1 has implications for physiological control and therapy. Somatostatin (SST) isoforms exist with 14 or 28 amino acids. Both isoforms of SST evoke endocytosis of the somatostatin receptor-2 (SSTR2), which is expressed throughout the enteric nervous system. After activation by SST14, SSTR2 recycles, whereas after activation by SST28, SSTR2 remains in endosomes, from where it may continue to signal [136]. This difference is attributable to differential susceptibility of the

SST isoforms to degradation by ECE1. ECE1 degrades SST14 in endosomes, which destabilizes the SST14-SSTR2-ARRB complex, allowing the receptor to recycle [136, 137]. Since ECE1 does not degrade SST28, SSTR2 remains in endosomes. Although metabolically stable SST analogs (e.g., octreotide) are effective treatments for several disorders [138], they have side effects in the gastrointestinal tract (constipation, cramps, nausea). Stable SST analogs that are resistant to ECE1 evoke prolonged sequestration of SSTR2 in enteric neurons, which may generate long-lasting signals that underlie beneficial and detrimental actions [136].

Mechanisms of Endosomal GPCR Signaling

The concept that endosomes are a major site for sustained GPCR signaling was suggested by observations that ARRBs serve as molecular scaffolds that recruit GPCRs and components of the mitogen-activated protein kinase cascades to endosomes [57, 126]. It is now apparent that GPCRs in endosomes can signal by ARRB- and G protein-mediated mechanisms, and that endosomal signaling activates kinases and generates cAMP in defined subcellular compartments [22-27, 118-121] (**Fig. 5**). How is it possible that GPCRs can signal from endosomes by ARRB- and G protein-mediated mechanisms, when ARRBs uncouple GPCRs from G proteins at the plasma membrane? Structural studies of the β_2-adrenergic receptor have identified receptor-G protein-ARRB megaplexes and revealed that conformations of GPCR-ARRB complexes retain the capacity to couple to G_α subunits [139, 140].

The Translational and Clinical Impact of GPCR Compartmentalized Signaling for Digestive Diseases

The therapeutic relevance of endosomal GPCR signaling is now evident [28]. Whereas GPCR signaling at the plasma membrane is transient, endosomal signaling by the same receptor can be sustained and regulate events in the cell, including gene transcription in the case of the β_2-adrenergic receptor and NK_1R [24, 121]. Endosomal signaling by GPCRs in the pain pathway, including the SP NK_1R and the CGRP calcitonin receptor-like receptor (CLR) in second order spinal neurons [24, 27], and PAR_2 in primary spinal afferent neurons [25], is critical for the sustained activation and hyperexcitability of neurons that is a hallmark of chronic pain. Indeed, receptor endocytosis is required for these receptors to exhibit the full repertoire of signaling responses. Inhibitors of clathrin and dynamin, and lipid-conjugated antagonists that target NK_1R, CLR and PAR_2 in endosomes block signaling derived from endosomal receptors. Such inhibitors provide

relief from pain in preclinical models of somatic and colonic pain [24, 25, 27] illustrating the pathophysiological relevance of endosomal GPCR signaling. Endosomally-targeted antagonists of PAR_2 may be effective treatments for IBS pain, in which colonic proteases and PAR_2 are strongly implicated [25, 141, 142]. Endosomally-targeted agonists and antagonists of GPCRs may provide options for therapy where this has proven to be clinically ineffective [28].

Future Directions

GPCRs control digestion and digestive diseases, and are a target for therapy. GPCRs sense the contents of the lumen, mediate the actions of gut hormones, neurotransmitters and paracrine agents, and control inflammation and pain. Drugs that activate or inhibit these receptors have been a mainstay for the treatment of digestive disorders (e.g., histamine H_2 receptor antagonists for peptic ulcer disease [143]).

However, we have but a superficial understanding of this large and complex family of receptors in digestion and digestive diseases. The functions and roles in the gut of orphan GPCRs, such as MRGPRs, leucine rich GPCRs, frizzled and adhesion receptors, are still unknown. The concepts of allosteric modulation, biased agonism, oligomerization and compartmentalized signaling offer new opportunities for therapy. The successful exploitation of these concepts for the development of superior therapies requires a complete understanding of receptor expression, signaling and trafficking in important cell types in health and diseased states, which is lacking.

Progress in structural, chemical and cell biology, and genetics will advance understanding of the function of GPCRs and the development of GPCR-directed therapies. Conventional drug discovery involves screens of libraries of millions of drug-like molecules. Although this approach has yielded success, some GPCRs have been found to be un-druggable. An understanding of the structural basis of GPCR activation and signaling, coupled with advances in molecular modeling, has enabled screening of virtual libraries in silico, allowing rational structure-based drug design, even for orphan GPCRs [144]. Cryo-electron microscopy [13, 14] and proximity ligation techniques coupled to mass spectrometry and proteomics [145] have provided fresh insights into the formation and structure of GPCR-signaling platforms. The realization that GPCRs can signal in defined subcellular compartments to control pathophysiologically important

processes, such as pain, has led to the development of compartment-selective agonists and antagonists [28]. Analysis of compartmentalized signaling using genetically-encoded biosensors has revealed that some drugs can activate GPCRs in unexpected intracellular locations. Whereas opioid peptides can activate MOR at the plasma membrane and then in endosomes, secondary to receptor endocytosis, morphine can also activate MOR in the Golgi apparatus, by virtue of its ability to penetrate membranes [54]. In this context, developments such as organoids, which replicate the complex organization of organs in tissue culture, and advanced genome editing using CRISPR Cas 9 hold remarkable potential in basic and translational GPCR research [146]. The development of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) and optogenetics have provided important insights into GPCR signaling pathways that underlie important physiological processes in vivo. DREADDs are engineered to respond to inert drugs, but not endogenous ligands. By using transgenic and viral-delivery approaches, it is possible to express DREADDS in particular cell types, and then examine the consequences of GPCR activation in defined cell types [147, 148]. Chemo-genetic approaches have been used to control the activity of enteric glial cells to investigate their roles in intestinal motility [149] and secretomotor function [150].

Much of the focus of these new technologies has been to define the function of GPCRs in the central nervous system and to develop more effective GPCR-directed therapies for neurological diseases. In light of the undoubted importance of GPCRs in the digestive system, the application of similar technologies to analysis of gut function may lead to advances in understanding digestive diseases.

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Author names in bold designate shared co-first authorship.

Figure Legends

Figure 1. GPCRs and their ligands in digestion and digestive disease. GPCRs are expressed throughout the digestive tract. Expression of some functionally and clinically important GPCRs in specific cell types in the tongue, lower esophageal sphincter, stomach, small intestine and colon are depicted. GPCRs control multiple processes in the gut and are targets for common diseases (e.g., GERD, gastric ulcer disease, disorders of intestinal motility, colonic pain and inflammation). T_xR, taste receptor; MGLURs, metabotropic glutamate receptor; $GABA_BR$, gamma-aminobutyric acid B receptor; H_xR, histamine receptor; M_xR, muscarinic acetylcholine receptor; EP3, prostaglandin receptor 3; SSTR, somatostatin receptor; $5HT_xR$, serotonin receptor; FFARs, free fatty acid receptors; P2YR, purinergic 2Y receptor; OR, opioid receptor; NKR, neurokinin receptor; PAR, protease-activated receptor; CBR, cannabinoid receptor; BKR, bradykinin receptor; CLR, calcitonin receptor-like receptor; RAMP1, receptor activity modifying protein 1; CCKAR, cholecystokinin A receptor; TGR5, Takeda G protein coupled receptor 5 bile-acid receptor; MRGPR, Mas-related G protein-coupled receptor; OTR, oxytocin receptor; VPR, vasopressin receptor.

Figure 2. Allosteric modulation of GPCRs. The orthosteric site of a GPCR is the site where the endogenous ligand (brown) binds. Sites that are topographically distinct from the orthosteric site are named

allosteric sites. Ligands that bind to allosteric sites (red) can potentiate or depress the orthosteric ligand affinity and efficacy and are named positive allosteric modulators (PAMs) or negative allosteric modulators (NAMs), respectively. The simulated concentration response curves show the effect of increasing concentrations of PAMs (green lines) or NAMs (red lines) on the response to a GPCR agonist (black line).

Figure 3. The therapeutic potential of biased agonists of GPCRs. Biased agonism describes the phenomenon whereby different ligands binding to the same GPCR in an identical cellular background elicit distinct signaling outcomes (pathway A and pathway B). Balanced agonists (ligand 1) are those that activate all signaling pathways to the same extent, leading to therapeutic effects but also to deleterious effects. When there is a distinction between the signaling pathways that drive a therapeutic response and those that mediate the adverse effects of a drug, biased agonists provide a novel avenue for pathway-directed therapeutics. In such a case, the drug would only trigger the desired response while sparing the unwanted, deleterious effects (ligand 2).

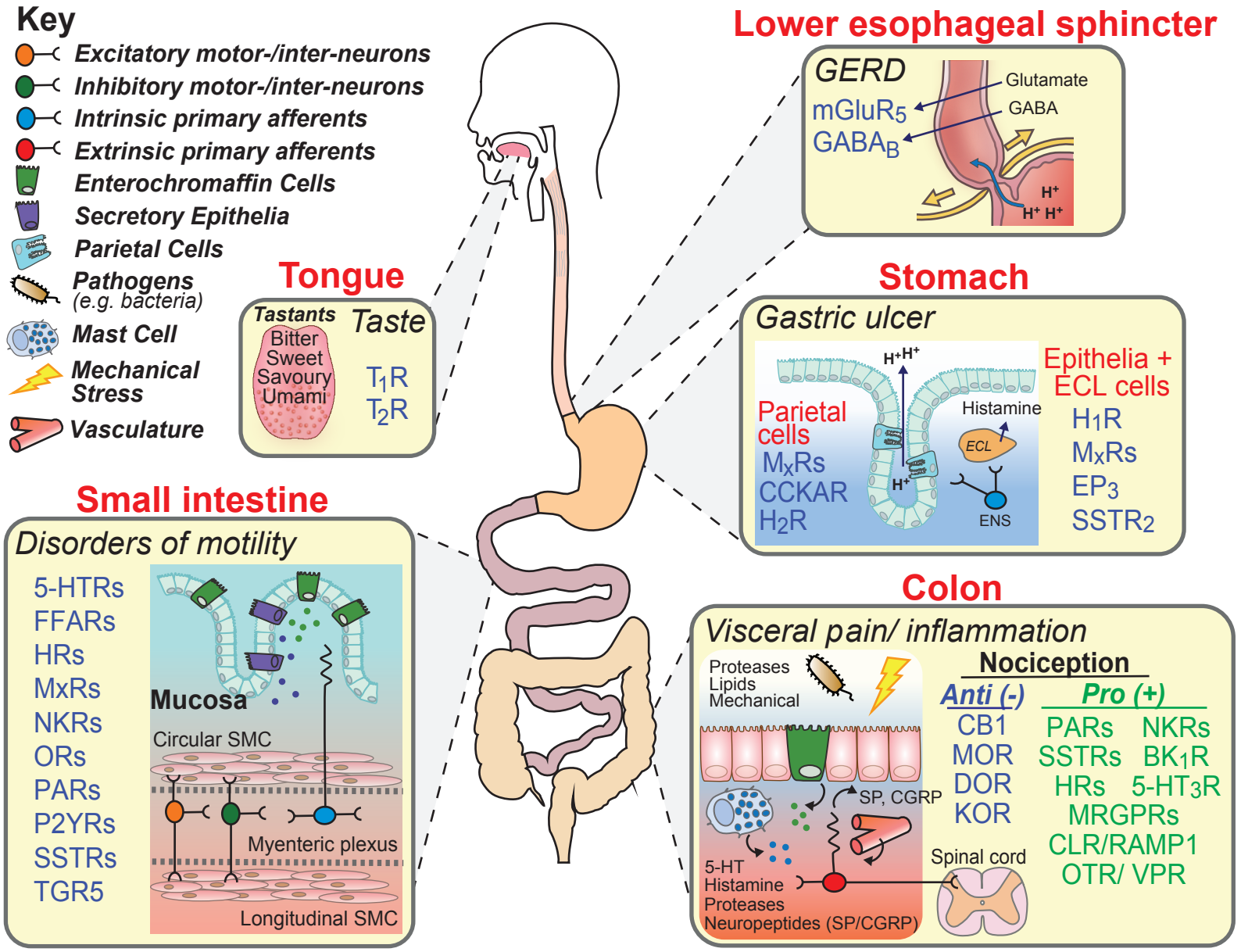
Figure 4. Potential roles of GPCR dimerization. GPCRs have been shown to function both as monomers (1) and dimers (2). The formation of GPCR dimers can be triggered by agonist activation and change the specificity of G protein coupling (3). Such differences in effector coupling elicited by dimerization have prompted the development of bivalent drugs, which specifically target both protomers within a dimer (4). Dimerization can also provide an alternative mechanism of receptor trafficking, whereby ligands can promote the co-internalization of both receptors after the stimulation of only one protomer (5). Alternatively, the presence of a protomer that is resistant to agonist-promoted endocytosis, within a heterodimer, can inhibit the internalization of the complex.

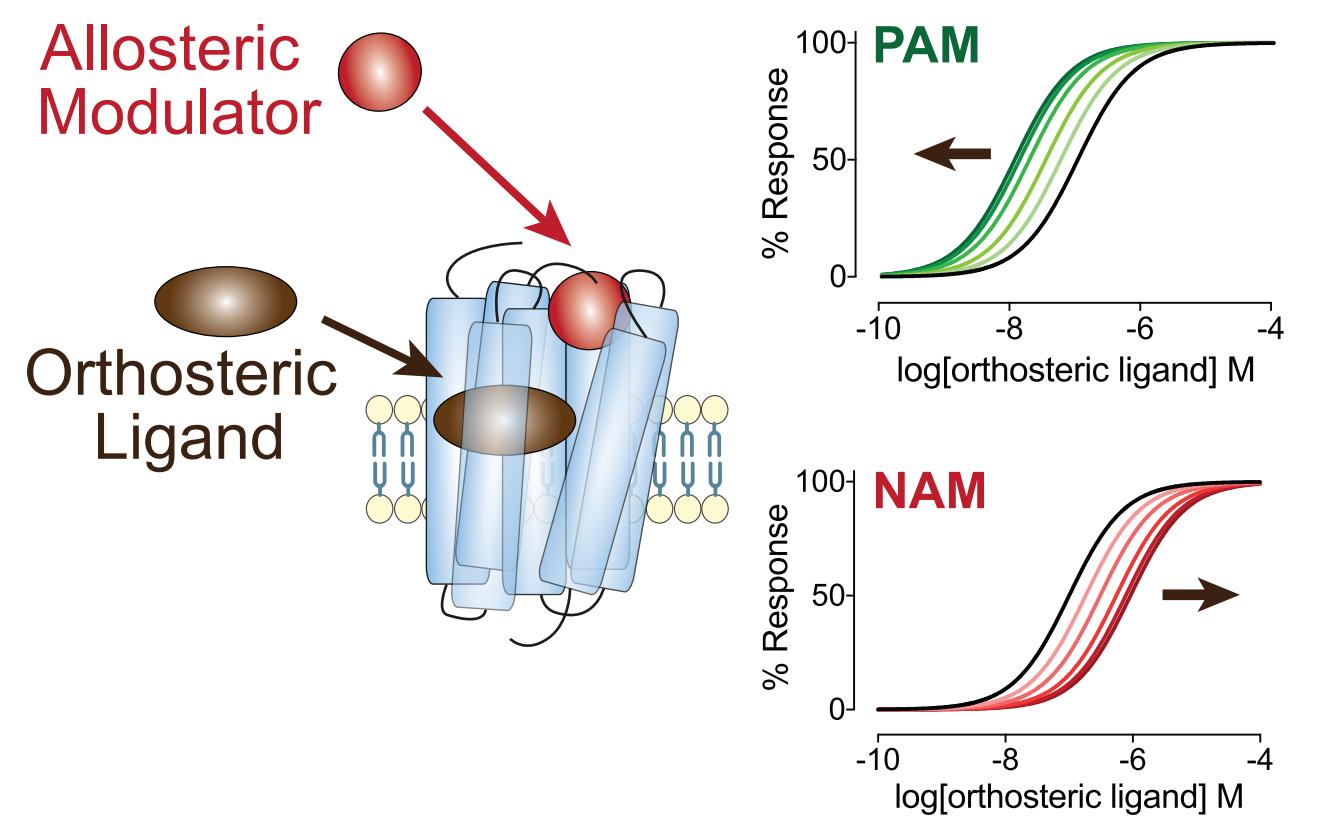
Figure 5. GPCR trafficking and compartmentalized signaling. The formation of GPCR-mediated signaling platforms provides a mechanism to sculpt specific cellular responses. (1) GPCRs at the plasma membrane form multiprotein complexes that participate in the regulation of a specific signaling pathway (pathway A). For example, AKAP (A-kinase anchor protein) interactions with GPCRs can scaffold the formation of complexes that regulate cAMP signaling by bringing in close proximity enzymes that degrade cAMP (phosphodiesterases, PDEs) and kinases that are activated by this second messenger (protein kinase A, PKA). (2) Upon prolonged agonist stimulation, GPCRs are phosphorylated by G protein receptor kinases

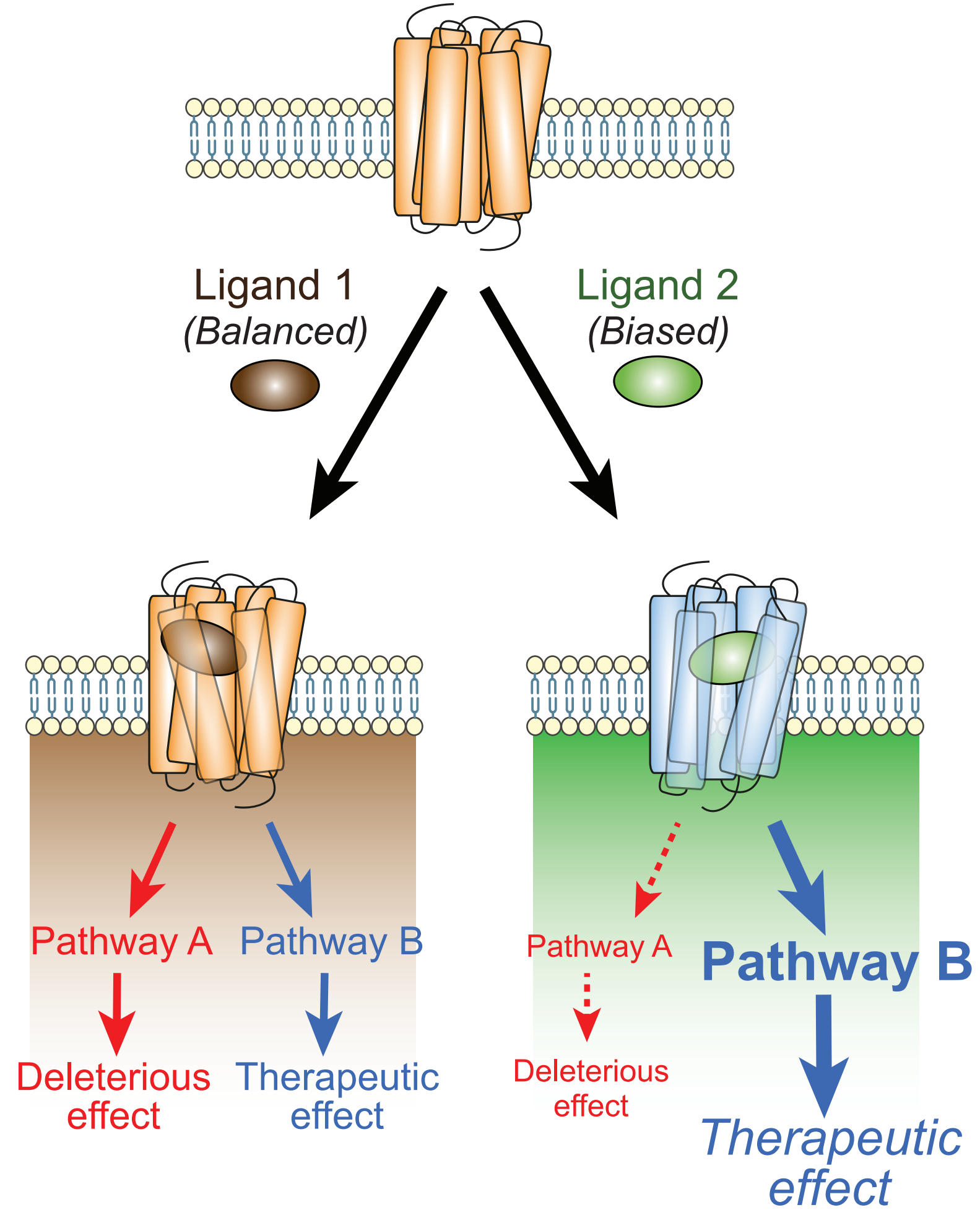
(GRKs). The phosphorylated receptor has higher affinity for the cytosolic protein ARRB. (3) ARRBs are adaptors that promote clathrin- and dynamin-mediated endocytosis of GPCRs. (4) ARRBs scaffold the formation of multiprotein complexes that result in a second wave of intracellular signaling (pathway B). Genetically encoded biosensors have revealed differences in the spatial and temporal profile of GPCR signaling from different subcellular locations (insets).

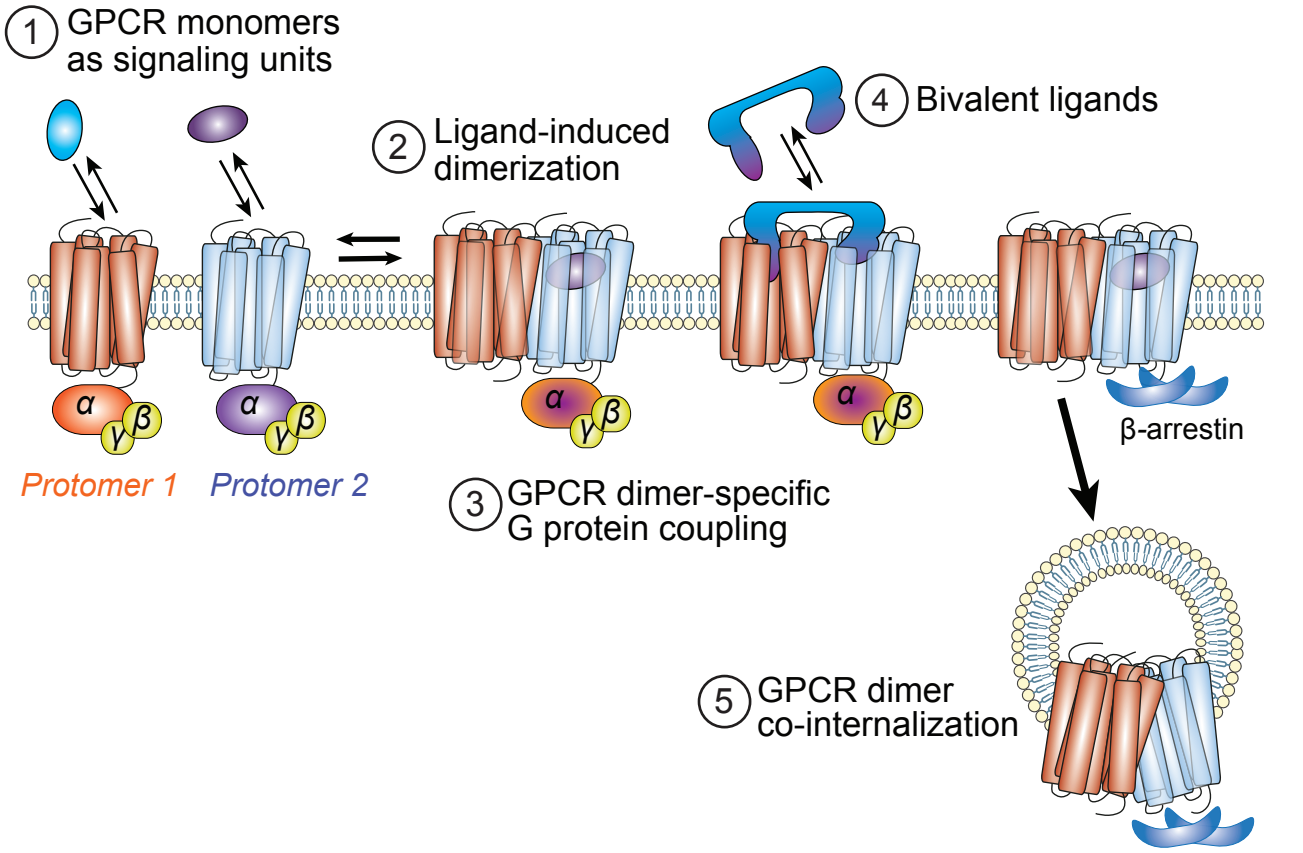
Table 1. Clinical trials of allosteric modulators, biased agonists and bivalent ligands of GPCRs for the treatment of disorders of the gastrointestinal tract or with side effects in the gastrointestinal tract.

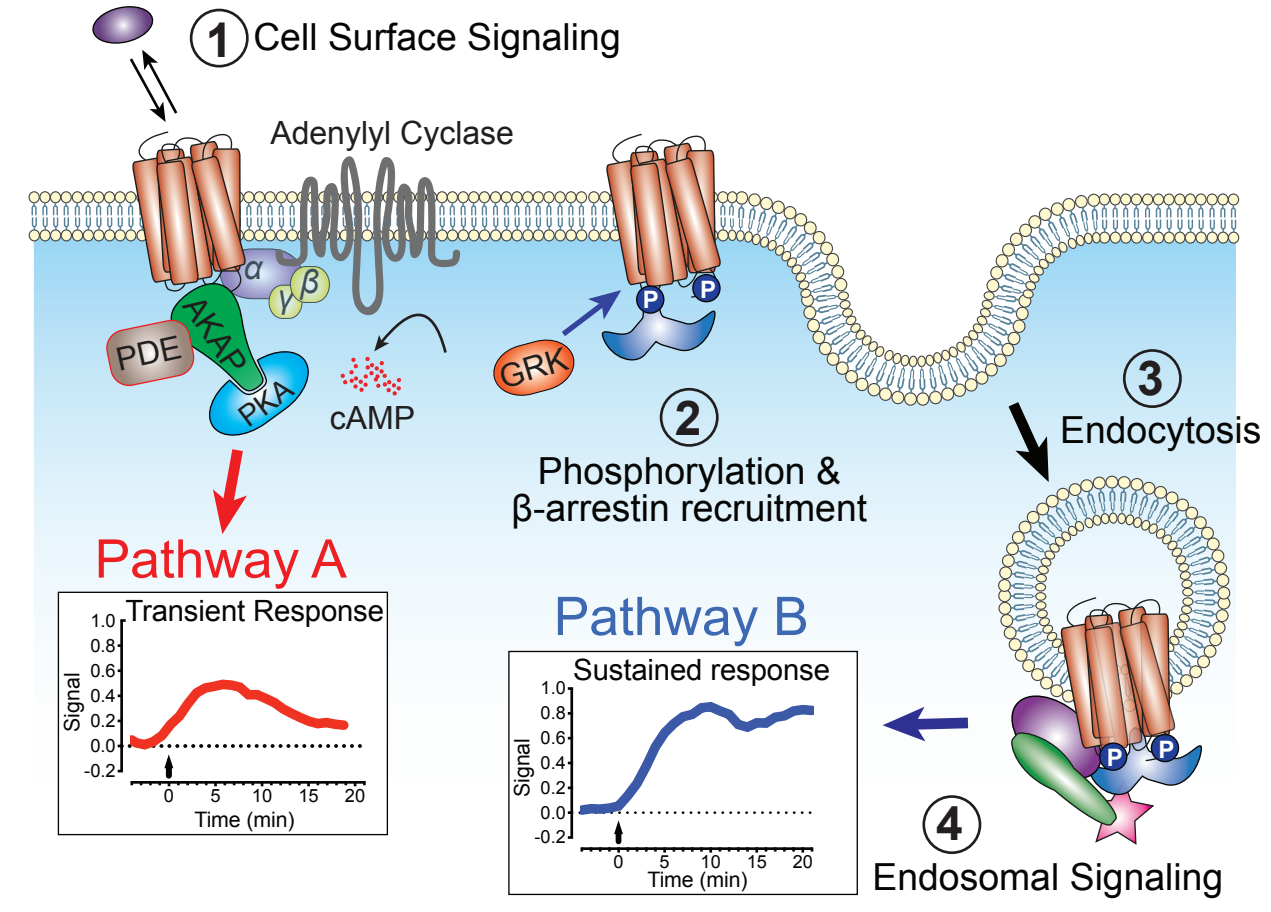
Drug	Mechanism of action	Clinical indication	Potential GI effect	Outcome of trial	ClinicalTrial.gov identifier
Allosteric modulators					
MK-7622	M_1R PAM	Improved cognition in Alzheimer's	Diarrhea	Trial stopped for futility; diarrhea most common side effect	NCT01852110
ADX10059	MGLUR5 NAM	GERD	Reduced reflux	Further testing stopped due to elevated hepatic transaminases	NCT00820079
Biased agonists					
TRV130	MOR agonist	Pain	Decreased nausea and vomiting; constipation not measured in trials	Analgesia comparable or better than morphine	NCT02335294 NCT02083315
ADL5859	DOR agonist	Pain	Possible reduced impact on GI motility relative to MOR agonist; however, not measured in trial	No analgesia	NCT00993863 NCT00626275 NCT00603265 NCT00979953
ADL5747	DOR agonist	Pain	Possible reduced impact on GI motility relative to MOR agonist; however, not measured in trial	Not effective for analgesia	NCT00979953 NCT01058642
Oligomer-targets					
Eluxadoline	MOR agonist and DOR antagonist	IBS-D abdominal pain	Analgesia for abdominal pain	Approved for clinical use	NCT01553747 NCT01553591
Eluxadoline	MOR agonist and DOR antagonist	IBS-D with bile acid malabsorption	Improved stool consistency	Recruiting	NCT03441581
Eluxadoline	MOR agonist and DOR antagonist	Diarrhea-associated fecal incontinence	Reduced days with fecal incontinence	Recruitment pending	NCT03489265


Key
Excitatory motor-/inter-neurons
Inhibitory motor-/inter-neurons
Intrinsic primary afferents
Extrinsic primary afferents
Enterochromaffin Cells
Secretory Epithelia
Parietal Cells
Pathogens
(e.g. bacteria)
Mast Cell
Mechanical Stress
Vasculature
Lower esophageal sphincter
GERD
mGluR5
GABAB
Glutamate
GABA
H+
H+ H+
Tongue
Tastants
Bitter
Sweet
Savoury
Umami
Taste
T1R
T2R
Stomach
Gastric ulcer
H+H+
Parietal cells
MxRs
CCKAR
H2R
Histamine
ECL
H+
ENS
Epithelia + ECL cells
H1R
MxRs
EP3
SSTR2
Small intestine
Disorders of motility
5-HTRs
FFARs
HRs
MxRs
NKRs
ORs
PARs
P2YRs
SSTRs
TGR5
Mucosa
Circular SMC
Myenteric plexus
Longitudinal SMC
Colon
Visceral pain/ inflammation
Proteases
Lipids
Mechanical
SP, CGRP
5-HT
Histamine
Proteases
Neuropeptides (SP/CGRP)
Spinal cord
Nociception
Anti (-)
CB1
MOR
DOR
KOR
Pro (+)
PARs
NKRs
SSTRs
BK1R
HRs
5-HT3R
MRGPRs
CLR/RAMP1
OTR/ VPR


Allosteric Modulator
Orthosteric Ligand
PAM
% Response
100
50
0
-10
-8
-6
-4
log[orthosteric ligand] M
NAM
% Response
100
50
0
-10
-8
-6
-4
log[orthosteric ligand] M


Ligand 1
(Balanced)
Ligand 2
(Biased)
Pathway A
Pathway B
Deleterious effect
Therapeutic effect
Pathway A
Pathway B
Deleterious effect
Therapeutic effect


1 GPCR monomers as signaling units
2 Ligand-induced dimerization
3 GPCR dimer-specific G protein coupling
4 Bivalent ligands
5 GPCR dimer co-internalization
α
β
γ
Protomer 1
Protomer 2
β-arrestin


1 Cell Surface Signaling
Adenylyl Cyclase
α
β
γ
AKAP
PDE
PKA
cAMP
GRK
P
P
2
Phosphorylation &
β-arrestin recruitment
3
Endocytosis
Pathway A
Transient Response
Signal
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
5
10
15
20
Time (min)
Pathway B
Sustained response
Signal
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
5
10
15
20
Time (min)
4
Endosomal Signaling