Receptors and Transmission in the Brain-Gut Axis: Potential for Novel Therapies

III. \( \mu \)-Opioid receptors in the enteric nervous system

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Sternini, Catia. Receptors and Transmission in the Brain-Gut Axis: Potential for Novel Therapies. III. \( \mu \)-Opioid receptors in the enteric nervous system. Am J Physiol Gastrointest Liver Physiol 281: G8–G15, 2001.—G protein-coupled receptors are cell surface signal-transducing proteins, which elicit a variety of biological functions by the activation of different intracellular effector systems. Many of these receptors, including the \( \mu \)-opioid receptor (\( \mu \)OR), have been localized in the gastrointestinal tract. \( \mu \)OR is the target of opioids and alkaloids, potent analgesic drugs with high potential for abuse. \( \mu \)OR is expressed by enteric neurons, and it undergoes ligand-selective endocytosis. It is of clinical importance because it mediates tolerance and other major side effects of opiate analgesics, including impairment of gastrointestinal propulsion. An important observation of \( \mu \)OR is its differential trafficking and desensitization properties in response to individual agonists, which might have long-term physiological consequences and be involved in the development of opiate side effects. Receptor activation by agonists is the basis for signaling, and alterations of the mechanisms controlling cellular responses of G protein-coupled receptors to agonists might be the basis of several diseases, including gastrointestinal diseases. Therefore, understanding these basic cellular mechanisms is important for developing appropriate therapeutic agents.

G protein-coupled receptors; receptor endocytosis; motoneurons; receptor trafficking

A VARIETY OF CHEMICAL MESSENGERS, including classic transmitters and numerous peptides, modulate multiple digestive functions, including motility, secretion, and absorption. These effects are mediated by the activation of specific receptors, most of which belong to the superfamily of seven-transmembrane G protein-coupled receptors. G protein-coupled receptors represent a large and versatile class of cell surface signal-transducing proteins, which activate different effector systems to induce a variety of biological functions (46). Biological responses to activated G protein-coupled receptors are regulated by receptor desensitization and resensitization, which are mediated by a cascade of events induced by ligand-receptor interaction and which govern cellular responsiveness to agonist stimulation (3). Desensitization, which refers to the diminution of agonist effect after stimulation, is a mechanism to prevent uncontrolled response of the cell to stimuli, whereas resensitization represents the process by which cells become responsive again to stimuli. These regulatory mechanisms are of clinical importance because they control signaling, and defects in their regulation may result in uncontrolled cellular response and diseases. Among the many events induced by ligand-receptor interaction, receptor endocytosis is of particular interest because it contributes to the regulation of receptor-mediated signal transduction by removing receptors from the cell surface and provides a means to identify sites of ligand release and neuronal circuits activated by a ligand.

G protein-coupled receptors comprise receptors for hormones and transmitters, including peptides (46). Peptides represent a major group of signaling molecules that exert many biological effects in the enteric nervous system. They can act as primary transmitters by directly exciting target cells, as cotransmitters by contributing to the excitability of the effector, as modulators by influencing the excitability of the target cell, or as growth factors (14). The molecular cloning of peptide receptors and the availability of peptide receptor antibodies have allowed the definition of the specific cell targets of peptides, which is essential to the elucidation of their sites and modes of action, but have also provided a valuable tool to establish whether these receptors are functional by visualizing their translocation from the cell surface to the cytoplasm after agonist stimulation. Several studies have investigated the endocytic and sorting pathway of G protein-coupled
receptors in transfected cells, and an increasing number of studies have also investigated these processes in highly differentiated cells including neurons. Many peptide receptors have been identified in neuronal and nonneuronal structures of the gastrointestinal tract. For instance, the receptors for tachykinins have been localized to enteric neurons as well as to smooth muscle cells and interstitial cells of Cajal (17, 43) and have been shown to undergo endocytosis in vitro and in vivo in response to exogenous ligands and to tachykinins endogenously released in response to stimuli (18, 37, 38). The focus of this themes article is on μ-opioid receptor (μ-OR) distribution, activation, and trafficking in the enteric nervous system. μ-OR is of clinical importance because it is the main mediator of the opioid-induced impairment of gastrointestinal transit, one of the many side effects of opiate drugs, and it mediates the development of tolerance and drug addiction that limit the usefulness of these therapeutic compounds (26, 35). μ-OR is expressed by functionally distinct enteric neurons, and it undergoes agonist-selective receptor endocytosis in enteric neurons in vivo and in vitro (40, 41). Agonist selectivity plays an important role in internalization, which in turn might influence the biological actions of μ-OR ligands, including opiate alkaloids, important therapeutic drugs commonly used in humans for pain control.

**OPIOID RECEPTORS AND THEIR LIGANDS**

Opioid receptors constitute an important group of G protein-coupled receptors that mediate the effects of endogenous opioid peptides and of structurally distinct alkaloid opiate drugs in the nervous system, including the enteric nervous system (25, 35). Three major receptors mediate opioid and opiate effects, the δ-, κ-, and μ-opioid receptors, which are distinguished by their affinity for opioids and alkaloids (35). Opioid receptors have specific pharmacological profiles and physiological functions, maintain a certain degree of selectivity for various opioid ligands, and display unique patterns of expression in the nervous system, even though there is overlap in their binding affinity, distribution, and function (34, 35). For instance, endorphins bind to μ- and δ-receptors with similar affinity, whereas dynorphins display some selectivity for κ-opioid receptor and enkephalins are the preferred ligands for δ- but also have remarkable affinity for μ-opioid receptors (9). By contrast, the recently discovered opioid peptides, endomorphin-1 and -2, which have been isolated from the brain (49), have the highest affinity and selectivity for μ-OR of any opioid peptides described to date. Endomorphins have several thousand-fold preference for μ-OR over δ- and κ-receptors. They have affinity for μ-OR similar to that of [d-Ala₂,N-Me-Phe⁶,Gly-ol⁸]enkephalin (DAMGO), one of the most potent μ-OR-selective enkephalin analogs, but far greater selectivity. Furthermore, these peptides have potent biological effects that mimic those of other μ-OR agonists (49). Endomorphin-1 induces analgesia in mice with a higher potency than morphine, and, like morphine and DAMGO, endomorphin-1 and endomorphin-2 inhibit electrically induced contraction of the guinea pig ileum (28, 44).

Unlike the well-established endogenous opioids, most alkaloids used clinically preferentially activate the μ-OR (34). Opiate alkaloids, which include morphine and fentanyl, are the most efficacious and potent analgesics used in humans for pain treatment. They are very important therapeutic drugs with high potential for abuse. They have been extensively studied because of their profound effects on the nervous system. Alkaloids exert a multitude of biological actions that include analgesia, respiratory depression, and inhibition of intestinal transit and secretion (24). Gene targeting studies have provided direct evidence for functional roles of the μ-OR in several biological activities of opiates including analgesia and inhibition of gastrointestinal transit, which are altered in μ-OR knockout mice (27, 36).

Three known families of opioid peptides that are endogenous to mammals have been reported in the gastrointestinal tract, where they are localized either in the enteric nervous system or in chromaffin cells (6). Specifically, in the enteric nervous system, the derivatives of proenkephalins are mostly confined to myenteric neurons projecting to the circular muscle and submucosal plexus (16), whereas prodynorphin-derived peptides are localized to submucosal and myenteric neurons and to fibers originating from the celiac ganglion (39). Opioids and opiates affect a variety of functions within the digestive system, including motility, transit, secretion, and electrolyte and fluid transport. The involvement of opioids in the control of contraction and propulsion is supported by a large body of anatomic and functional evidence (19, 20, 24). Opioid peptides and alkaloids impair intestinal transit in humans and other mammalian species by changing the coordinated reflex motor activity into a segmenting and nonpropulsive motility pattern (24, 25). In the guinea pig, which has been used extensively as a model for functional studies to characterize the physiological and pathophysiologic effects of opioids, morphine-induced blockade of propulsion and transit is not associated with muscular spasm (24, 25). In the gastrointestinal tract, opioid receptor binding sites corresponding to the three classes of opioid receptors have been associated with different structures including smooth muscle and neurons (6, 24). However, it appears that the neural effects of opioids in intact tissues are more relevant than their direct effects on muscle (6). To date, morphological studies using specific antibodies for the cloned opioid receptors have provided direct evidence for the presence of μ- and κ-opioid receptors in the enteric nervous system (1, 41). Furthermore, functional and pharmacological evidence indicates that the effects of opioids on intestinal motility induced by the activation of opioid receptors on neuronal structures are predominantly mediated by μ- and κ-opioid receptors (21, 25).
**μ-OPIOID RECEPTOR DISTRIBUTION IN THE ENTERIC NERVOUS SYSTEM**

μOR has been reported in enteric neurons of the rat and guinea pig gastrointestinal tract. In the rat, μOR has been observed in neurons of both the submucosal and myenteric plexus and in fibers distributed to the muscle, vasculature, and mucosa, as well as in presumed interstitial cells of Cajal in the myenteric plexus and deep muscular plexus (1, 42). By contrast, in the guinea pig, μOR immunoreactivity is primarily localized to neurons of the myenteric plexus, which are more abundant in the small intestine, particularly the ileum, than in the stomach and colon, and to fibers distributed to the interconnecting strands and smooth muscle layer, where they form a dense network in the deep muscular plexus. μOR enteric neurons have the morphological characteristics of Dogiel type I myenteric neurons, with an oval cell body, many thick dendrites protruding from the soma, and a long axonal process, which can be followed within the plexus and between plexuses in the interconnecting strands (41). Dogiel type I neurons comprise motoneurons that transmit information to the muscle cells and control smooth muscle activity and interneurons that transmit information to other enteric neurons (15). μOR-immunoreactive neurons represent a large population of myenteric neurons, roughly corresponding to 30% of myenteric neurons in the guinea pig ileum. μOR immunoreactivity is predominantly localized at the cell surface in nonstimulated conditions, and it translocates to endosomes after activation with selective μOR ligands (Refs. 40 and 41; see below). The μOR immunoreactivity distribution closely matches the distribution of the opioid peptide enkephalin (16). Indeed, fibers containing enkephalin immunoreactivity are in some cases in close vicinity to myenteric neurons bearing μOR. In addition, μOR and enkephalin immunoreactivities colocalize in some myenteric neurons and fibers distributed to the circular muscle and the deep muscular plexus, where they often surround interstitial cells of Cajal (unpublished observations). Enkephalins are capable of activating μOR and of triggering μOR endocytosis in enteric neurons and are likely to be the endogenous opioids that primarily activate neuronal μOR in the gut, even though they are not selective for this opioid receptor type (35). It is possible that endomorphins also act as endogenous ligands for the μOR in the enteric nervous system, because these novel opioids activate and induce internalization of μOR in enteric neurons in vitro (28). However, there is a lack of evidence for these peptides in the gastrointestinal tract.

μOR myenteric neurons comprise functionally distinct types of neurons. These include cholinergic and tachykinergic ascending, excitatory motoneurons to the muscle, as demonstrated by the presence of substance P and choline acetyltransferase (ChAT), a marker for cholinergic neurons, in a large proportion of μOR myenteric neurons (unpublished observations). They also likely include ascending interneurons, which contain the same combination of chemical messengers as excitatory motoneurons (10). In addition, μOR enteric neurons comprise a large proportion of descending neurons, as indicated by the localization of nitric oxide synthase, the enzyme that synthesizes nitric oxide (NO), and vasoactive intestinal polypeptide (VIP), the major markers of descending inhibitory motoneurons (10, 14), as well as cholinergic descending interneurons, as indicated by their coexpression of ChAT and VIP.

The presence of μOR on cholinergic ascending excitatory neurons that innervate the muscle is consistent with the functional evidence that opioids and opiates inhibit the electrically evoked release of acetylcholine by acting on enteric neurons primarily via the μOR. This in turn results in inhibition of muscle contraction, which is responsible for the delayed gastrointestinal transit and severe constipation induced by opioids (24). Opioid receptors and ligands might also modulate acetylcholine's effect on motor activity, as suggested by the hypersensitivity of ascending reflexes to acetylcholine in morphine-tolerant animals (24). On the other hand, the presence of μOR on a substantial population of VIP/NO descending neurons is in agreement with a modulatory effect of opioids on the release of VIP and the production of NO. Reduced release of inhibitory transmitters would account for the reported excitatory effect of opioids on smooth muscle (2). This is also in agreement with the opioid inhibitory effect on compliance of the intestinal wall during the preparatory phase of peristalsis in the intact segment of the guinea pig ileum (45). Indeed, because μOR agonists do not appear to act directly on the muscle of the guinea pig small intestine (21) and they inhibit enteric neurons (33), μOR agonist-induced inhibition of compliance of intestinal wall resistance could be attributed to direct activation of inhibitory motoneurons to the circular muscle and/or to a reduction of the excitability of interneurons located in the reflex pathway. Inhibition of VIP release and NO production could also suppress descending relaxation and consequently interfere with intestinal propulsion (19, 20). Together, these findings support the hypothesis that the opioid effects on intestinal transit mediated by the activation of μOR result from the inhibition of selected enteric neurons and that opioids act by modulating transmitter release from neurons of the ascending and descending pathways.

**μ-OPIOID RECEPTOR ACTIVATION AND TRAFFICKING**

μOR is an inhibitory G protein-coupled receptor functionally coupled to several effector pathways, including inhibition of adenyl cyclase and of cAMP formation, increase of potassium currents, inhibition of calcium currents, modulation of inositol trisphosphate turnover, and activation of mitogen-activated protein kinase (11, 32, 35). Coupling of the μOR to these effector systems attenuates neuronal activity by inhibiting neurotransmitter release and changing neuronal excitability by pre- and postsynaptic mechanisms. After ligand-receptor interaction, μOR undergoes adap-
tations such as desensitization, downregulation, and resensitization in response to agonist treatment. These events regulate cellular responsiveness to receptor activation and result from receptor-mediated processes including phosphorylation, receptor endocytosis, intracellular sorting, and recycling (3).

The presence of μOR at the cell surface is essential for cellular activation, and agonist-induced receptor internalization plays an important role in regulating cellular responsiveness by depleting the cell surface of receptors and by contributing to the process of resensitization (3). μOR undergoes rapid, ligand-induced internalization in transfected cells as well as in neurons (22, 23, 41). In the enteric nervous system, μOR internalization occurs in both the soma and neuronal processes; it is prevented by the opioid receptor antagonist naloxone (41) and by the selective μOR antagonist 6-p-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP) (40), and it persists for 4–6 h. μOR internalization in enteric neurons is concentration dependent, and it occurs predominantly via a clathrin-mediated mechanism. After appropriate intracellular sorting requiring endosomal acidification, μOR recycles to the cell surface at ~6 h (unpublished observations). Rapid μOR internalization in enteric neurons is triggered by opioids, including enkephalins (unpublished observations) and endomorphins in vitro (40), and by opiates such as etorphine and fentanyl in vitro and in vivo (41) (Fig. 1). By contrast, morphine, a high-affinity μOR agonist, does not induce μOR endocytosis in enteric neurons under the same experimental conditions, suggesting that different mechanisms regulate cellular responsiveness to opioid ligands. Indeed, an interesting and intriguing aspect of μOR trafficking and signaling is that opioid agonists with similar abilities to activate μOR signaling have remarkably different abilities in inducing μOR internalization and in functionally desensitizing the μOR (22, 23, 28, 41, 47, 48, 50). Unlike etorphine and opioid peptides that induce rapid μOR internalization and desensitization, morphine, which is the prototype of opiate analgesics and which activates the same intracellular pathways as other opiates (35), fails to induce μOR internalization and desensitization, even at saturating concentrations far in excess of those inducing maximal inhibition of electrically induced muscle contraction (40) and inhibition of cAMP formation (22). Morphine does not trigger μOR endocytosis in enteric neurons, but it partially inhibits μOR endocytosis induced by the opiate alkaloid etorphine, providing evidence that it activates and occupies μORs (41). This agonist selectivity of μOR endocytosis that we have observed in enteric neurons (28, 40, 41) has been confirmed in neurons of the central nervous system and in cell lines transfected with μOR cDNA (22, 23), showing that it is a generalized phenomenon of this receptor. This agonist-selective μOR trafficking provides the basis for the hypothesis that individual agonists might have differential abilities to regulate biological effects mediated by the μOR. This would be in agreement with the observation that the ability to induce tolerance is inversely correlated with opiate effectiveness in inducing endocytosis, i.e., opiates that trigger μOR endocytosis appear to be less prone to induce tolerance compared with morphine, which does not induce receptor endocytosis under the same experimental conditions (12).

μOR internalization in enteric neurons also occurs in response to endogenously released opioids. Indeed, electrical stimulation of longitudinal muscle-myenteric plexus preparations with frequencies within the range that has been shown to induce enkephalin release (8) is a potent stimulus for evoking μOR internalization in enteric neurons (40). There is a relationship between stimulus intensity and level of internalization. Low

![Fig. 1](http://ajpgi.physiology.org/)

**Fig. 1.** μ-Opiate receptors (μOR) on enteric neurons in unstimulated (A) and stimulated (B and C) conditions. μOR immunoreactivity is predominantly confined at the cell surface in unstimulated (A) neurons. Enteric neurons have the morphology of Dogiel type I with a long axonal process (arrow) and many thick dendrites protruding from the soma (arrowheads). B and C: μOR immunoreactivity in endosomes after stimulation with either DAMGO (B), an enkephalin analog, or etorphine (C). Shown are confocal images of enteric neurons from organotypic cultures of the intestine incubated with DAMGO (1 μM) for 60 min at 4°C to allow ligand-receptor binding, washed, and either fixed immediately (A) or incubated at 37°C in ligand-free solution for 30 min to allow receptor endocytosis (B). C shows μOR in endosomes after exposure to 100 nM etorphine.
frequency induces low levels of internalization in a few neurons, whereas high frequency results in high levels of internalization in many neurons. These findings clearly indicate that native μORs endocytose after activation by endogenously released opioids. Thus μOR endocytosis can serve as an indication of opioid release and also can be used as a means to visualize neuronal pathways activated by endogenous opioids.

An important component of μOR function and regulation in the gastrointestinal tract is the presence of spare μORs. Spare μORs are functional receptors that can be activated by agonists. The existence of spare μORs implies that an agonist can exert its full effect by activating only a fraction of the total μOR population on the cell surface (7). The greater the excess of functional μORs in a system, the lower is the concentration of agonist required for an effect to occur. The μOR reserve in the guinea pig myenteric plexus is substantial, comprising ~90% of the total μOR population; therefore, only 10% occupancy is required for μOR agonist effects to occur. The level of opioid receptor reserve is important in the regulation of cellular sensitivity to opioids and opiates (7). The magnitude of μ-opioid spare receptor fraction appears to control the potency of individual opioid ligands, and alteration of this fraction has been proposed as one of the putative mechanisms of opioid tolerance. We have provided evidence that reduction of μ-opioid spare receptor reserves together with ligand-induced μOR endocytosis might serve as mechanisms to regulate μOR responsiveness to stimulation. This proposal is based on our observation that ligand-induced μOR endocytosis in enteric neurons reduces the nerve-mediated response (i.e., electrically induced muscle twitch contraction and acetylcholine release) in vitro in neuromuscular preparations of the small intestine in which μ-opioid spare receptors have been inactivated by pretreatment with an alkylating agent, β-chlornaltrexamine (β-CNA), at concentrations that irreversibly inactivate opioid receptor reserve (40). However, the reduction of neurogenic response by ligand-induced μOR endocytosis was not observed in preparations in which the receptor spare fraction was not reduced. This indicates that the reduction of μ-opioid spare receptors plays an important role in the diminution of the nerve-mediated response to opioids induced by μOR endocytosis. On the other hand, the observation that concentrations of morphine that completely abolish electrically induced muscle contractions but fail to induce receptor endocytosis do not affect the subsequent muscle twitch response to increasing concentrations of morphine, even in conditions in which spare receptors have been inactivated, provides strong support for the proposal that receptor endocytosis is an important element in the attenuation of cellular responsiveness to agonist stimulation. These studies clearly indicate that both receptor endocytosis and reduction of spare receptor fraction contribute to the diminution of neuronal responsiveness to opioids.

Studies on cell lines have shown that etorphine and opioid peptides induce μOR phosphorylation and plasma membrane translocation of β-arrestins followed by dynamin-dependent receptor internalization (50), whereas morphine's failure to induce desensitization is accompanied by a lack of μOR phosphorylation and translocation of β-arrestins with consequent lack of receptor internalization. However, morphine induces μOR phosphorylation with β-arrestin translocation and receptor sequestration when G protein kinase 2 is overexpressed (50). This suggests that alterations of the machinery involved in receptor trafficking might play an important role in the regulation of cellular signaling.
responsiveness to μOR agonists. Because activation and internalization of native μOR are comparable to that observed in cell lines, it is reasonable to assume that these processes described in transfected cells also occur in highly differentiated cells. Figure 2 schematically illustrates what occurs to μOR after activation with ligands capable of triggering receptor endocytosis. Activated μORs are phosphorylated by G protein receptor kinases, they bind β-arrestins, and they endocytose via a dynamin-dependent mechanism that involves clathrin-coated pits (47) as other protein-coupled receptors. In addition to phosphorylate-activated receptors, G protein receptor kinases promote interaction with β-arrestins, which block agonist-mediated signal transduction by uncoupling receptors from G proteins, therefore inducing desensitization (13). β-Arrestins also act as adaptor proteins for dynamin-dependent clathrin-mediated endocytosis, linking the receptor to the endocytic machinery, and regulate the rate at which endosomal receptors are dephosphorylated and recycled, therefore contributing to the resensitization process. Dynamin is a cytosolic GTPase that regulates the formation and internalization of clathrin-coated vesicles and mediates early endosome formation (29). Dynamin is required for the internalization of many G protein-coupled receptors, including the μOR (47, 50). These intracellular proteins play an important role in the regulation of μOR function. Indeed, overexpression or disruption of β-arrestins in cell lines affects μOR trafficking and signaling. Overexpression of β-arrestins as well as overexpression of G protein receptor kinase 2 facilitate endocytosis of morphine-activated μOR (47, 50), and functional deletion of β-arrestin 2 gene induces remarkable potentiation and prolongation of morphine-induced analgesia and prevents the occurrence of μOR desensitization and tolerance with chronic morphine treatment (4, 5). Furthermore, overexpression and translocation of dynamin from intracellular pools to plasma membranes have been observed after chronic treatment with morphine (31), suggesting that dynamin upregulation may be an important component of the increased neuronal plasticity that has been recognized at the basis of morphine addiction (30). Alterations of intracellular regulatory proteins involved in receptor trafficking are hypothesized to play a role in the development of tolerance and perhaps other important side effects of opiate drugs. In addition, the magnitude of the μ-opioid spare receptor fraction appears to control the potency of individual opioid ligands, and alteration of this fraction might be one of the mechanisms of opioid tolerance.

CONCLUSION AND FUTURE PROSPECTS

Since the first discovery, a few years ago, that individual opioid ligands that activate μOR through the same signaling pathways differ in their ability to induce rapid endocytosis of μOR, there has been increasing interest in trying to understand the physiological implications of this dissociation between receptor signaling and endocytosis. This, together with the observation that the ability of opioid agonists to induce receptor internalization is inversely correlated with their efficacy to induce tolerance (12), supports the concept of ligand-specific effects on intracellular adaptations induced by alkaloids. In addition, it is becoming evident that different μOR ligands form different receptor conformations, that there are differences within the μOR binding domains among agonists, and that ligand-specific effects on intracellular proteins involved in receptor trafficking may occur. Together, these findings have forced a reevaluation of the mechanisms underlying μOR activation and regulation (47). The high analgesic potency of opiate alkaloid drugs is limited by the development of important side effects including tolerance, dependence, respiratory depression, and profound impairment of gastrointestinal transit often resulting in severe constipation. Therefore, an elucidation of the mechanisms responsible for these side effects is of clinical importance for the development of therapeutic agents that preserve their efficacy as analgesics but are less effective in inducing tolerance and other side effects. A better knowledge of the roles and functional implications of receptor-mediated processes will provide the basis for the development of novel therapies targeted to the specific processes that affect neuronal responsiveness. For instance, μOR endocytosis might play a more complex role than being involved in the regulation of signal transduction and in the maintenance of cellular desensitization by controlling cell surface receptor expression and might also regulate physiological responses and biological actions of agonists. This is particularly important for its clinical implications, suggesting that μOR endocytosis might influence the therapeutic action of potent analogues like the opiate alkaloids. The concept that receptor endocytosis might regulate physiological and pathophysiological processes, as proposed for the μOR, can also be applicable to other G protein-coupled receptors. It is reasonable to speculate that general biological roles of receptor endocytosis will be identified for other G protein-coupled receptors and that ligand-induced receptor endocytosis is involved in biological and adaptive responses mediated by activated G protein-coupled receptors.

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