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REGULATION OF G PROTEIN-COUPLED RECEPTORS BY PHOSPHORYLATION AND ENDOCYTOSIS

MARK VON ZASTROW

G protein-coupled receptors (GPCRs) comprise a large superfamily of heptahelical integral membrane proteins that mediate transmembrane signal transduction in response to a wide variety of hormones, neurotransmitters, and neuromodulators. GPCRs are extremely important targets for neuropsychopharmacology. Indeed, the vast majority of clinically relevant neuropsychiatric drugs either bind directly to specific GPCRs (e.g., many antipsychotic drugs) or function indirectly via GPCRs by influencing the amount of available native agonist (e.g., many antidepressant drugs).

A general feature of GPCRs is that they are extensively regulated in cells (1–4). Regulation of GPCRs is thought to play a fundamental role in maintaining physiologic homeostasis in the face of fluctuating internal and external stimuli. A number of pathologic states are associated with disturbances in the number or functional activity of certain GPCRs (5). In addition, many clinically important drugs influence the physiologic regulation of GPCRs (6). Together, these observations suggest that mechanisms of GPCR regulation may be of fundamental importance to neuropsychiatric disorders and to the actions of clinically relevant drugs.

The physiologic and biomedical importance of GPCR regulation has motivated an enormous amount of study into underlying molecular mechanisms of regulation. Progress in this area has been facilitated enormously by molecular and cell biological approaches applied to a variety of experimental model systems. Our understanding remains at an early stage and is limited, in most cases, to studies of a small number of GPCRs. Nevertheless, great progress has been made in elucidating certain mechanisms of GPCR regulation, to the extent that it is possible to begin to discern fundamental principles that control the number and functional activity of GPCRs in individual cells.

The present chapter discusses some of this progress, with an emphasis on developing a unified view of GPCR regulation. We have restricted our scope to a limited number of regulatory mechanisms that have been elucidated by detailed study of the some of the most extensively characterized GPCRs. First, we survey classic studies describing the general properties of the physiologic and pharmacologic regulation of receptor-mediated signaling; these have established a terminology and conceptual framework for our later focus on specific mechanisms of receptor regulation. Second, we discuss pioneering studies of "prototypic" GPCRs that have established paradigms for understanding the role of receptor phosphorylation in mediating rapid desensitization of GPCRs. Third, we focus on a specific mechanism mediating regulated endocytosis of certain GPCRs, and discuss how this endocytic mechanism can promote rapid desensitization and resensitization of receptor-mediated signal transduction. In this section, we also highlight the close interdependence between mechanisms of GPCR phosphorylation and membrane trafficking in mediating rapid regulation of receptor function. Finally, we discuss the functions of both phosphorylation and endocytic membrane trafficking in mediating longer-term regulation of the number of GPCRs present in cells, focusing on recent studies into mechanisms that control down-regulation of receptors via proteolytic degradation in lysosomes.

GENERAL PROCESSES OF GPCR REGULATION

Rapid Desensitization and Resensitization

It has been known for many years that multiple mechanisms can contribute to GPCR regulation (1,7). Early studies,

Mark von Zastrow: Department of Psychiatry, Department of Cellular and Molecular Pharmacology, and Program in Cell Biology, University of California, San Francisco, California.

which preceded the elucidation of any of the biochemical machinery involved, distinguished general processes of receptor regulation according to differences in kinetics and reversibility. This is well illustrated by classic studies of the β_2-adrenergic receptor (B2AR), reviewed in detail elsewhere (2–4). Agonist-induced activation of the B2AR stimulates adenylyl cyclase via coupling to the G_s heterotrimeric G protein (Fig. 1A). Receptor-mediated signaling via this pathway occurs within seconds after agonist binding. However, after more prolonged activation, the ability of receptors to activate adenylyl cyclase via G_s diminishes greatly. This diminution of signal transduction is generally called *desensitization* and is mediated, at least in part, by regulation of the receptor itself. A process of *rapid desensitization* was so named because it occurs within seconds to minutes after agonist-induced activation. Rapid desensitization of the B2AR can be reversed within several minutes after removal of agonist in a process called *resensitization.* Rapid desensitization of the B2AR is not associated with a decrease in the total number of receptors present in cells or tissues, and resensitization does not require biosynthesis of new receptor protein. Therefore, rapid desensitization is thought to reflect a change in the *functional activity,* rather than absolute number, of receptors (Fig. 5.1B).

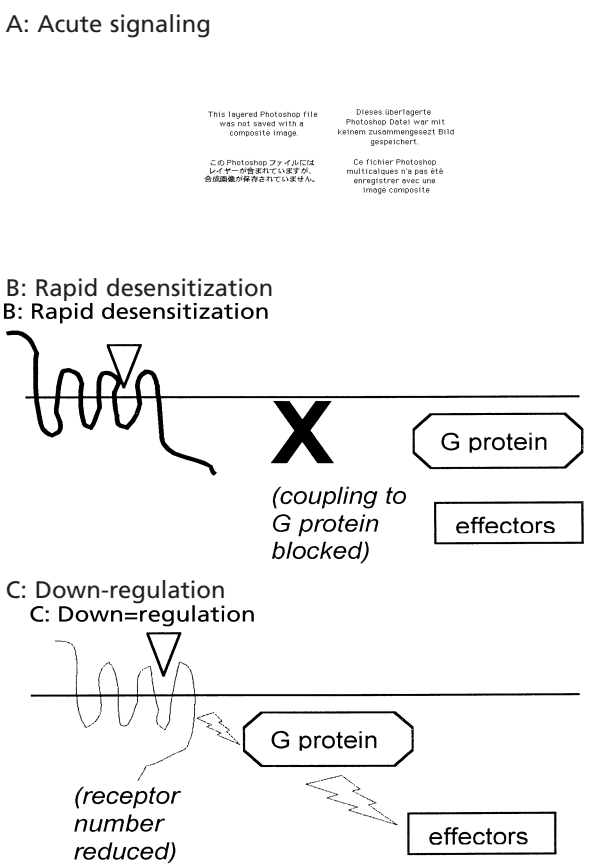

FIGURE 5.1. Desensitization and down-regulation of G protein-coupled receptors. **Panel A:** Within milliseconds to seconds after agonist binding, receptors present in the plasma membrane mediate signal transduction to effectors by functionally coupling (promoting guanine nucleotide exchange on) a heterotrimeric G protein. **Panel B:** Within several minutes after agonist binding, rapid desensitization occurs by functional uncoupling of the receptor from G protein. This represents a change in the functional activity of receptors, which inhibits signal transduction to the effector without changing the number of receptors in the cell. **Panel C:** After more prolonged agonist-induced activation of receptors (typically several hours to days), the number of receptors present in cells is greatly reduced, so signal transduction via G proteins to effectors is strongly attenuated. This process is called *receptor down-regulation* because it is thought to represent primarily a reduction in the number, rather than functional activity, of receptors.

Down-regulation and Up-regulation of Receptors

The kinetics of rapid desensitization and resensitization may be relevant to the physiologic action of catecholamines (endogenous agonist ligands for the B2AR), as these molecules can be released intermittently by vesicular exocytosis and are rapidly removed from the extracellular milieu by membrane transport, enzymatic degradation, or both. However, many clinically important drugs that activate GPCRs have a more prolonged duration of action. Studies of these drugs established the existence of a distinct process of receptor regulation that occurs much more slowly, typically within several hours to days after prolonged or repeated exposure of tissues to ligand. This process is called *down-regulation* because (in contrast to rapid desensitization) it is associated with a pronounced decrease in the total number of receptors present in cells or tissues, as typically detected by means of radioligand binding techniques. Further distinguishing the process of down-regulation from rapid desensitization, recovery of signaling activity after down-regulation is generally a slow process that requires biosynthesis of new receptor protein (8). Therefore, down-regulation is thought to reflect primarily a change in the *number,* rather than functional activity, of receptors present in cells or tissues (Fig. 5.1C). In most cases, down-regulation of GPCRs (like rapid desensitization) is induced by *agonists,* but not by antagonists. Moreover, certain antagonists can induce an opposite process of increased receptor number called *up-regulation* (9, 10). These observations are consistent with a fundamental role of down-regulation and up-regulation as a negative feedback mechanism that seeks to maintain physiologic homeostasis of receptor signaling. However, in some cases, processes associated with GPCR down-regulation may be induced by antagonists (11,12). In other cases, up-regulation of receptors can be induced by drugs with partial agonist activity (10). These observations suggest that certain clinically relevant drugs may not simply mimic or block the effects of endogenous agonists. Indeed, it is proposed that such ''paradoxical'' regulatory effects may contribute to the pathologic or therapeutic actions of certain clinically rele-

vant neuropsychiatric drugs, including opiate analgesics and atypical antipsychotic agents (10,13–16).

Distinguishable Processes of Homologous and Heterologous Desensitization

Another important observation leading to our present view of GPCR regulatory mechanisms has come from studies investigating the pharmacologic specificity of receptor regulation (1,17). Many cell types express multiple types of GPCR (Fig. 5.2A). It has been observed that in some cases, prolonged activation of one type of GPCR causes attenuated signal transduction only by that receptor, without any detectable effect on signaling by other types of GPCR present in the same cell. In this case, the regulation of receptors is said to be *homologous* (Fig. 5.2B). The existence of homologous processes of regulation provided early evidence, before specific regulatory mechanisms were elucidated, that signal transduction can be modulated by modification of the receptor itself. In other cases, activation of one type of GPCR attenuates signaling not only by that receptor but also by other type(s) of GPCR present in the same cell. Regulation of this kind is said to be *heterologous* (Fig. 5.2C). Heterologous regulation of receptor signaling is consistent with modification of a "downstream" component in the signal transduction pathway that is involved in signaling by more than one type of GPCR. However, as discussed below, important examples of heterologous regulation mediated by modification of the receptor protein itself also exist. Homologous processes of receptor regulation are capable of modulating signal transduction in a highly specific manner, whereas heterologous processes of regulation may play an important role in facilitating functional "cross-talk" between pharmacologically distinct signaling pathways (18).

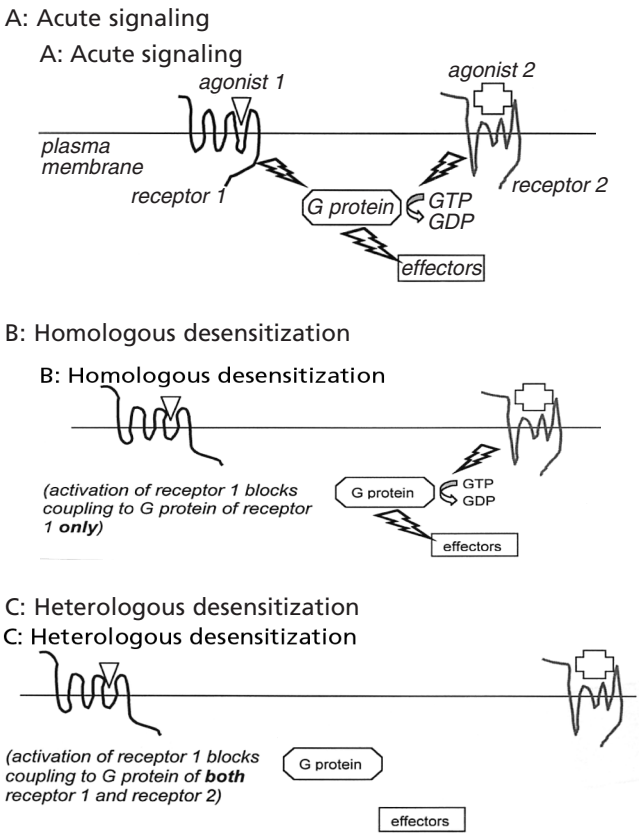

FIGURE 5.2. Homologous and heterologous desensitization. **Panel A:** Receptors 1 and 2 can signal via the same G protein-mediated pathway. **Panel B:** Agonist 1 causes uncoupling only of receptor 1; signaling induced by agonist 2 binding to receptor 2 is not affected. This is homologous desensitization of receptor 1. **Panel C:** Agonist 1 causes functional uncoupling of both receptor 1 and receptor 2, so that signaling induced by both agonists is blocked. This is heterologous desensitization.

SPECIFIC MECHANISMS OF GPCR REGULATION

Rapid Desensitization Mediated by Phosphorylation-Dependent Uncoupling of Receptor from Heterotrimeric G Protein

It is well established that many GPCRs are regulated by phosphorylation. Classic studies of rhodopsin (a light-activated GPCR) and the B2AR (a ligand-activated GPCR) provide examples of illustrating distinct molecular mechanisms that mediate homologous and heterologous desensitization of receptors. Because the principles behind these mechanisms have proven to be widely applicable to other GPCRs, rhodopsin, and the B2AR are often considered "prototypic" GPCRs that have established general paradigms for understanding phosphorylation-dependent regulation of GPCRs (19).

Phosphorylation of Rhodopsin: a Model for Functional Inactivation of GPCRs

Elegant studies of the vertebrate visual system identified a critical role of phosphorylation in inactivating rhodopsin following light-induced activation (20). Light-activated rhodopsin is a good substrate for phosphorylation by a cytoplasmic enzyme called *rhodopsin kinase,* whereas rhodopsin that has not been activated by light is a poor substrate (20). Phosphorylation of the carboxyl-terminal cytoplasmic domain of rhodopsin is sufficient to attenuate the ability of light-activated rhodopsin to couple functionally to its cognate heterotrimeric G protein (transducin). Studies of rhodopsin function in isolated membrane fractions indicated that rhodopsin kinase-mediated phosphorylation can strongly attenuate rhodopsin signaling, but it does so to a lesser extent than observed in the intact rod cell. A second cytoplasmic protein was identified that, when added to membrane preparations in combination with rhodopsin kinase, greatly accelerates the attenuation of rhodopsin signaling (21). This protein was proposed to act as a protein

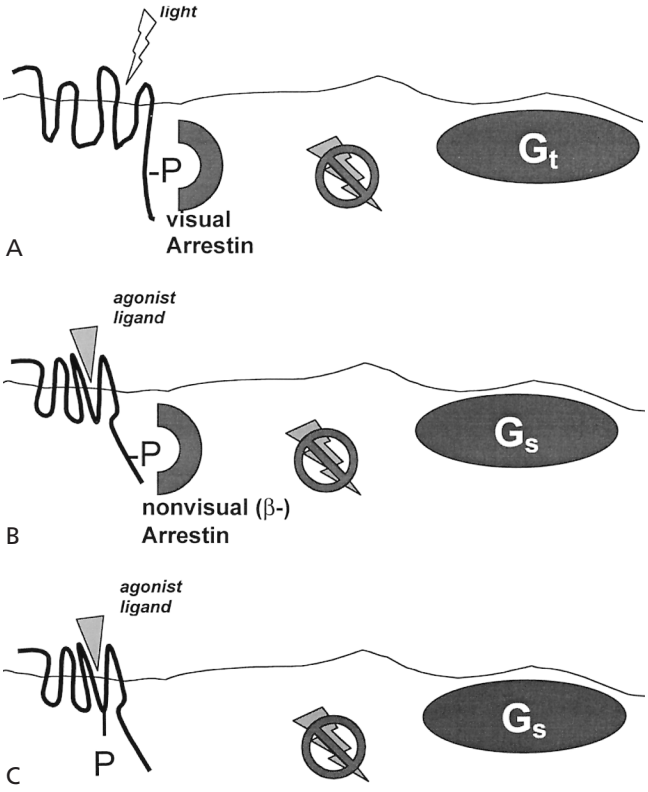

FIGURE 5.3. Paradigms for phosphorylation-dependent desensitization. **A:** GRK1-mediated inactivation of rhodopsin. **B:** GRK2-mediated desensitization of the B2AR. **C:** PKA-mediated desensitization of the B2AR.

cofactor that "arrests" signal transduction by phosphorylated rhodopsin and was therefore called *arrestin* (Fig. 5.3A).

GRK-Mediated Phosphorylation of the B2AR: a Model for Homologous Desensitization

Studies of functional reconstitution of B2AR-mediated activation of adenylyl cyclase provided compelling evidence for a role of phosphorylation in mediating rapid desensitization of a ligand-activated GPCR (22). Biochemical purification of the cytoplasmic activity responsible for this phosphorylation identified a kinase called β-*adrenergic receptor kinase* (BARK), and it was noted that this kinase preferentially phosphorylates agonist-occupied receptors (23). Protein sequencing and cyclic DNA (cDNA) cloning later indicated that this kinase is closely similar in structure to rhodopsin kinase (24). Furthermore, cDNA-cloning experiments in which reduced stringency hybridization was used identified additional kinases homologous to rhodopsin kinase and BARK (19). Collectively, these observations led to the discovery of the family of *G protein-coupled receptor kinases* (GRKs). According to this nomenclature, rhodopsin is denoted GRK-1, the originally identified BARK enzyme is denoted GRK-2, and other members of this family of protein kinases are numbered sequentially thereafter. Six members of the GRK family of receptor kinases have been identified to date.

Biochemical reconstitution studies indicated that increasingly purified fractions of BARK exhibit a reduced ability to attenuate B2AR-mediated signal transduction. Further analysis of this effect led to the identification of a distinct protein component that copurifies with BARK in initial stages of purification but is resolved from BARK in more highly purified fractions. This protein component reconstitutes strong attenuation of B2AR-mediated activation of adenylyl cyclase when added back to highly purified fractions of BARK (25,26). The protein cofactor involved in desensitization of the B2AR turned out to be a protein similar to visual arrestin and was therefore named β-*arrestin* or *barrestin.* cDNA cloning has identified additional proteins with similar structure, thus defining a family of protein cofactors for phosphorylation-dependent regulation of GPCR function (4). Two nomenclatures are currently in common use for these molecules. In one, the originally identified β-arrestin is denoted barrestin 1, and additional homologues are named sequentially barrestin 2, and so on. In another nomenclature, all members of this protein family are referred to as *arrestins,* with visual arrestin denoted arrestin 1, β-arrestin as arrestin 2, and subsequently identified family members numbered sequentially thereafter. Four members of the arrestin family of protein cofactors have been identified to date.

As noted above, an important feature of many GRKs is that their kinase activity is highly sensitive to the conformation of the receptor that they phosphorylate. This property of GRKs facilitates specific phosphorylation of only those receptors that are activated by ligand, whereas other receptors present in the same cells (but not activated by agonist) are not phosphorylated. Thus, GRK-mediated phosphorylation is generally considered to be a paradigm for *homologous* desensitization (Fig. 5.3B).

Protein Kinase A-Mediated Phosphorylation of the B2AR: a Model of Heterologous Desensitization

Other kinases, such as the so-called second messenger-regulated kinases, are also implicated in mediating desensitization of GPCRs. For example, the B2AR can be phosphorylated by cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA). PKA-mediated phosphorylation of a single residue located in the third intracellular loop of the B2AR impairs the ability of the receptor to couple to G_s and subsequently activate adenylyl cyclase (27–29). Phosphorylation of this residue is thought to impair receptor–G protein coupling directly, without a requirement for any known protein cofactor such as an arrestin. An important feature of PKA is that this kinase can phosphorylate B2ARs whether or not they have been acti-

vated by ligand, in contrast to the preferential phosphorylation of agonist-activated receptors by GRK-2. Because PKA is activated by cAMP, a signaling intermediate produced as a result of B2AR activation, PKA-mediated phosphorylation of the B2AR can be viewed as an example of feedback inhibition by a second messenger. In addition, because activation of any other receptor that stimulates adenylyl cyclase can also activate PKA, phosphorylation of the B2AR by PKA is generally considered to be a paradigm for *heterologous* desensitization (Fig. 5.3C).

Rapid Desensitization and Resensitization Mediated by Regulated Endocytosis of Receptors

Endocytosis of GPCRs by Clathrin-Coated Pits

Studies conducted in cultured cells indicate that many GPCRs can be regulated by ligand-induced endocytosis. The B2AR is perhaps the most extensively studied GPCR with respect to endocytic membrane trafficking. Early evidence for agonist-induced endocytosis of the B2AR was suggested by observations from subcellular fractionation and radioligand binding assays conducted with membrane-impermeant antagonists (30,31). These studies indicated that the number of B2AR binding sites detected in the plasma membrane can be reduced within several minutes after agonist-induced activation, a process called *sequestration.*

The development of receptor-specific antibodies allowed the application of immunocytochemical methods to visualize the subcellular localization of the B2AR and directly demonstrate agonist-induced internalization of the receptor protein. Internalization of the B2AR was observed to represent a steady state of a highly dynamic process involving continuous endocytosis and recycling of receptors through an endocytic pathway similar to that mediating constitutive (ligand-independent) endocytosis of nutrient receptors (32). This dynamic cycling of the B2AR was also suggested by elegant studies in which subcellular fractionation and radioligand binding techniques were used (33).

Regulation of B2AR endocytosis was shown to be mediated by a ligand-dependent lateral redistribution of receptors in the plasma membrane, from a relatively diffuse distribution throughout the plasma membrane to a pronounced concentration of agonist-activated receptors in structures resembling clathrin-coated pits when examined by immunoelectron microscopy. Furthermore, this process of ligand-regulated concentration of B2ARs in coated pits of the plasma membrane was shown to be mechanistically distinct from the subsequent endocytosis of receptors by membrane fission, which can occur even in the absence of continued ligand-induced activation of receptors (34). A protein that is required for this latter step of endocytic membrane fission is the cytoplasmic guanosine triphosphatase *dynamin* (35, 36). Consistent with this, agonist-induced endocytosis of the B2AR is inhibited by overexpression of certain "dominant-negative" mutant forms of dynamin (37,38). Subsequent studies have demonstrated that regulated endocytosis of several other GPCRs is also mediated by a dynamin-dependent mechanism, which suggests a conserved role of clathrin-coated pits in the endocytosis of many GPCRs (Fig. 5.4).

Role of GPCR Phosphorylation in Promoting Endocytosis by Clathrin-Coated Pits

Studies elucidating the mechanism mediating the key step of regulated concentration of GPCRs in clathrin-coated pits were initiated by the observation that GRK-mediated phosphorylation of the M2 muscarinic acetylcholine receptor can promote agonist-induced endocytosis of the receptor, whereas a kinase-defective mutant form of GRK inhibits this process (39). Similar observations were made for other GPCRs, including the B2AR (37,40). In an elegant series of

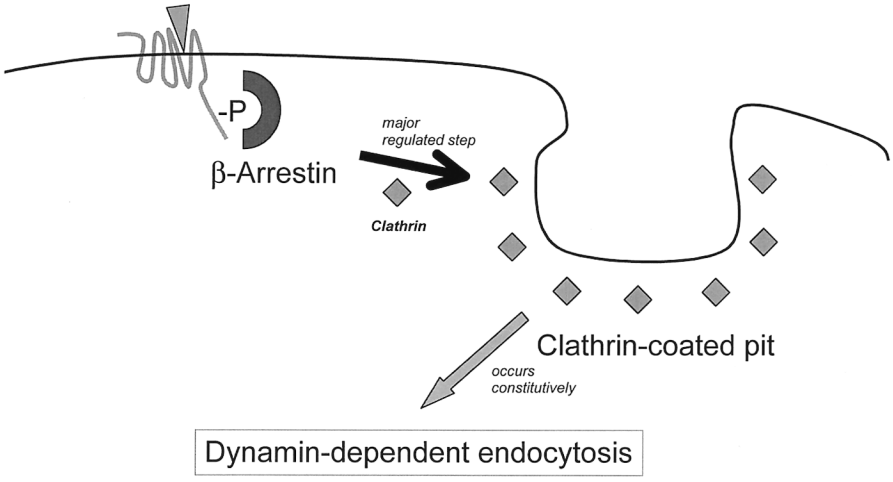

FIGURE 5.4. Regulated endocytosis of the β_2-adrenergic receptor (B2AR) by clathrin-coated pits. G protein-coupled receptor kinase-mediated phosphorylation of the B2AR promotes receptor interaction with nonvisual arrestins, which cause uncoupling of heterotrimeric G proteins and also promote interaction of arrestin–receptor complexes with clathrin coats. Once concentrated in clathrin-coated pits by this mechanism, receptors undergo endocytosis rapidly (even if agonist is removed from the receptor in the coated pit) via a constitutive (ligand-independent) mechanism of endocytic membrane fission that requires the cytoplasmic guanosine triphosphatase dynamin.

experiments, it was shown that certain arrestins can directly promote B2AR concentration in clathrin-coated pits by physically linking phosphorylated receptors with clathrin (40). This endocytic "adapter" function of arrestins can be distinguished from the function of arrestins as cofactors for functional uncoupling of receptor from G protein because the latter process can occur in the absence of endocytosis. Further distinguishing these functions, visual arrestin (arrestin 1) was shown to be unable to serve as an adapter for B2AR endocytosis even though it can serve as a cofactor for desensitization mediated by functional uncoupling of G protein (41). This distinction between visual and nonvisual arrestins led to the identification of a carboxyl-terminal clathrin-binding domain, present specifically in nonvisual arrestins, that is necessary for endocytosis of GPCRs but not for phosphorylation-dependent uncoupling of receptors from heterotrimeric G proteins (42,43).

Functional Role of Endocytosis in the Processes of Rapid Desensitization and Resensitization of GPCRs

Physiologic ligands are generally thought to bind to GPCRs in the plasma membrane. Biochemical and immunocytochemical studies suggest that certain GPCRs, such as the B2AR, interact with heterotrimeric G proteins primarily in the plasma membrane but not in intracellular membranes after endocytosis (44,45). Together, these observations suggest that endocytosis may, by itself, mediate *desensitization* of GPCR-mediated signal transduction by directly reducing the number of receptors present in the plasma membrane. Indeed, in some cases, endocytosis may be a principal mechanism of rapid desensitization (46). However, as discussed above, rapid desensitization by phosphorylation-dependent uncoupling of receptor from G protein does not require endocytosis of the receptor. This is consistent with the ability of certain GPCRs, such as the α_{2A}-adrenergic receptor, to desensitize in the absence of detectable endocytosis (47, 48). Studies of μ-opioid receptors expressed in transfected cells suggest that the effectiveness of endocytosis as a means of attenuating signal transduction is inversely proportional to the number of "spare receptors" present in cells (46). Thus, the precise role of endocytosis in contributing to desensitization of GPCR-mediated signal transduction probably varies among systems and may be particularly important in cells expressing relatively low numbers of receptors.

Strong evidence is available to indicate that endocytosis of certain GPCRs serves a distinct function in promoting *resensitization,* rather than desensitization, of signal transduction. The most thoroughly studied example of this mechanism derives from elegant studies of the B2AR (2, 49,50). As discussed above, agonist-induced phosphorylation of the B2AR by GRKs causes rapid desensitization by promoting receptor interaction with arrestins and functional uncoupling from heterotrimeric G proteins (Fig. 5.5, step 1). This initial desensitization of receptors occurs in the plasma membrane and does not require endocytosis of the receptor protein. Within several minutes after this initial uncoupling of receptor from heterotrimeric G protein, arrestins promote the concentration of receptors in clathrin-coated pits and subsequent endocytosis (Fig. 5.5, steps 2 and 3). Endocytic membranes containing internalized

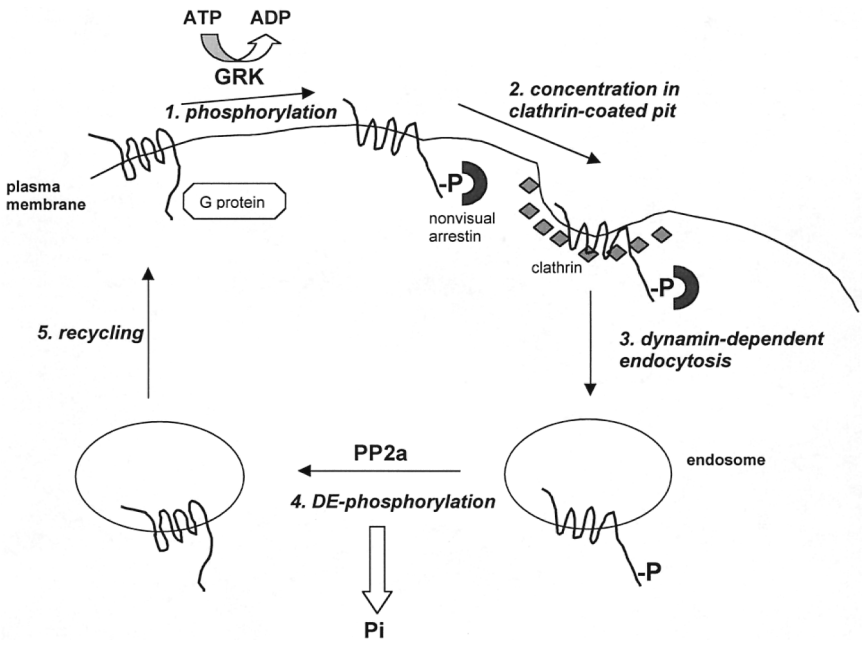

FIGURE 5.5. Linked cycles of G protein-coupled receptor phosphorylation and endocytosis mediating rapid desensitization and resensitization of the β_2-adrenergic receptor (B2AR). Agonist-induced activation of the B2AR causes G protein-coupled receptor kinase-mediated phosphorylation, which promotes receptor interaction with nonvisual arrestins and uncoupling of heterotrimeric G protein (step 1). Arrestin binding to the phosphorylated receptor also promotes receptor concentration in clathrin-coated pits (step 2), promoting rapid endocytosis of receptors by dynamin-dependent fission of coated pits from the plasma membrane and subsequent formation of endocytic vesicles (step 3). Together, these steps cause profound functional desenstization of signal transduction. Endocytic membranes containing internalized B2ARs are associated with a protein phosphatase (PP2A) that can catalyze dephosphorylation of receptors (step 4). Dephosphorylation of receptors followed by recycling to the plasma membrane (step 5) mediates the return of receptors to the plasma membrane in a fully functional state, promoting functional resensitization of the signal transduction system.

B2ARs are associated with activity of a protein phosphatase (PP2A) that can catalyze dephosphorylation of receptors (51). Based on these observations, it is proposed that endocytosis of receptors promotes dephosphorylation of receptors, after which receptors can be recycled back to the plasma membrane in a dephosphorylated, fully active state (Fig. 5.5, steps 4 and 5). Supporting this hypothesis, inhibitors of B2AR endocytosis do not block agonist-induced desensitization but strongly inhibit resensitization of receptor-mediated signal transduction following removal of agonist from the culture medium (52). Thus, agonist-induced regulation of the B2AR appears to involve two linked regulatory cycles: a biochemical cycle mediating phosphorylation and dephosphorylation of receptors, and a membrane trafficking cycle mediating endocytosis and recycling of receptors.

Down-regulation of GPCRs by Regulated Proteolysis

Evidence for Regulated Proteolysis of GPCRs

Down-regulation of GPCRs is defined from saturation binding analysis by a decrease in total specific binding sites (*B*max) without a change in apparent affinity (*K*d), which suggests that down-regulation reflects a decreased number of total receptors present cells or tissues (1,53). In principle, this process could be mediated by modulation of receptor biosynthesis or degradation. In practice, evidence suggests that both classes of mechanism contribute to the GPCR down-regulation observed physiologically. The role of transcriptional regulatory mechanisms in controlling biosynthesis have been characterized in some detail (see Chapter 17). As discussed in greater detail below, there is also considerable evidence supporting the importance of proteolysis of the receptor itself in mediating down regulation of a number of GPCRs (8).

Mechanisms of GPCR Proteolysis

Multiple mechanisms can mediate GPCR proteolysis. Proteolysis of endocytosed receptors in lysosomes is perhaps the best-established mechanism contributing to GPCR down-regulation in mammalian cells, including certain neurally derived cell types (54,55). However, it is apparent that other mechanisms of GPCR proteolysis also exist, some of which may not involve membrane trafficking of receptors at all. For example, the V2 vasopressin receptor can undergo ligand-induced proteolysis at the cell surface by a nonendocytic mechanism mediated by a plasma membrane-associated metalloprotease (56). Recent studies of B2AR down-regulation support the idea that regulated proteolysis of GPCRs can occur without endocytosis in some cell types (57).

In mammalian cells, ubiquitination is well established to promote degradation of various cytoplasmic proteins by a nonlysosomal mechanism mediated by proteasomes (58). Emerging evidence also suggests a role of ubiquitination in promoting endocytosis and proteolytic degradation of certain membrane proteins, including GPCRs in yeast (59, 60). The role of such a mechanism in mediating down-regulation of mammalian signaling receptors comes from studies of receptor tyrosine kinases (61). Alternate mechanisms of GPCR proteolysis in mammalian cells have been reported to be mediated by a distinct, nonproteasomal mechanism (56) or have been shown to be insensitive to inhibitors of proteasome-mediated proteolysis (57). Thus, to our knowledge, it is not yet clear to what extent ubiquitination or proteasomes may contribute to down-regulation of GPCRs in mammalian cells.

Membrane Pathway Mediating Receptor Delivery to Lysosomes

The delivery of membrane proteins from the plasma membrane to lysosomes is a multiple-step process that is mediated by endocytosis of receptors from the plasma membrane followed by shuttling to lysosomes via a specific series of membrane transport reactions (62,63). It is well-known that many GPCRs undergo ligand-induced endocytosis. However, specific mechanisms and pathways mediating subsequent stages of receptor trafficking to lysosomes are poorly understood.

Early studies demonstrated that ligand-induced sequestration and down-regulation of the B2AR can be differentially affected by pharmacologic manipulations and receptor mutation, which suggests that these processes may be mediated by distinct mechanisms (64–67). Furthermore, naturally occurring subtypes of α_2-adrenergic receptor down-regulate with similar rates (68) despite significant differences in their ability to undergo rapid endocytosis (47,48). Analogous processes of rapid sequestration and more gradual down-regulation have also been observed in studies of opioid receptors, where pharmacologic differences between the effects of individual agonists are very pronounced (69–71) and appear to be relevant to the physiologic effects of opiate drugs in native neurons (14,72). Compelling evidence for the existence of distinguishable membrane trafficking mechanisms comes from recent studies of mutant thrombin and substance P receptors, in which divergent residues in the carboxyl-terminal cytoplasmic domain specify differences in receptor trafficking between lysosomal and recycling pathways (73). Analyses based on kinetic modeling techniques are consistent either with completely separate pathways mediating rapid endocytosis and proteolytic degradation of GPCRs or with the operation of partially overlapping pathways that differ in their rate-limiting step (53). These models differ in whether sorting of GPCRs is proposed to occur before or after endocytosis (Fig. 5.6).

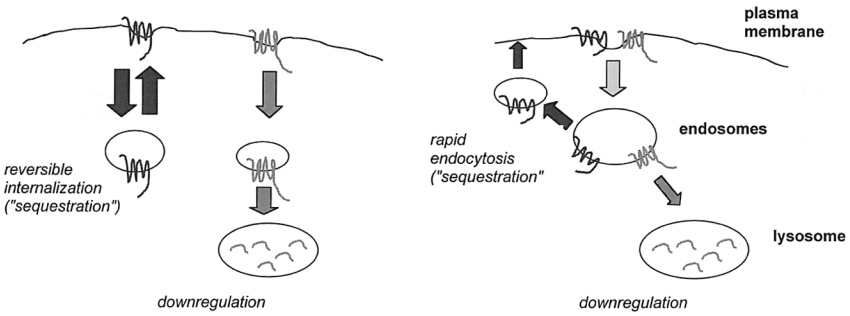

FIGURE 5.6. Models for sorting of G protein-coupled receptors (GPCRs) between pathways mediating reversible internalization (''sequestration'') and proteolytic down-regulation of GPCRs. **Panel A:** This model proposes that distinct GPCRs are segregated in the plasma membrane and are subsequently endocytosed by different membrane vesicles that either mediate reversible internalization or deliver receptors to lysosomes. **Panel B:** This model proposes that receptors can be endocytosed by the same membrane mechanism and delivered to the same early endocytic vesicles. Receptors are sorted after endocytosis between distinct pathways that mediate either recycling or lysosomal delivery of receptors.

Molecular Sorting of GPCRs after Endocytosis

Extensive previous studies of receptor-mediated uptake of extracellular ligands indicate that sorting of receptors between recycling and lysosomal pathways can occur after endocytosis (74). Recent studies of adrenergic and opioid receptors suggest that this is also true for certain GPCRs. For example, it has been shown recently that both agonist-induced sequestration and down-regulation of the B2AR are specifically inhibited by a dominant-negative mutant form of dynamin, which suggests that endocytosis of receptors by clathrin-coated pits is an obligate first step common to membrane pathways leading to recycling of endosomes and lysosomes (75). Recent studies of membrane trafficking of opioid receptors, which are also endocytosed by a dynamin-dependent mechanism involving clathrin-coated pits (76), support this conclusion. These studies demonstrate that distinct GPCRs can be sorted in a type-specific manner between recycling and degradative pathways after endocytosis by the same mechanism (Fig. 5.6B). These studies also suggest that the machinery that sorts GPCRs can function very rapidly (within several minutes) after the initial endocytosis of receptors (77).

Insight into a Mechanism Controlling Endocytic Sorting of the B2AR

The membrane machinery controlling postendocytic sorting of internalized GPCRs to lysosomes remains poorly understood. A recent study of B2AR trafficking has provided initial insight into a specialized mechanism that controls sorting of a specific GPCR between recycling and lysosomal pathways. As noted above, the B2AR is capable of recycling rapidly to the plasma membrane following endocytosis in cultured cells. It was observed that a series of mutations in the distal part of the carboxyl-terminal cytoplasmic tail strongly inhibit recycling of receptors and cause receptors to be ''mistargeted'' to lysosomes (78). All the receptor mutations that cause this phenotype disrupt a specific interaction of the B2AR with NHERF/EBP50/E3KARP-family proteins (78–80), a previously described

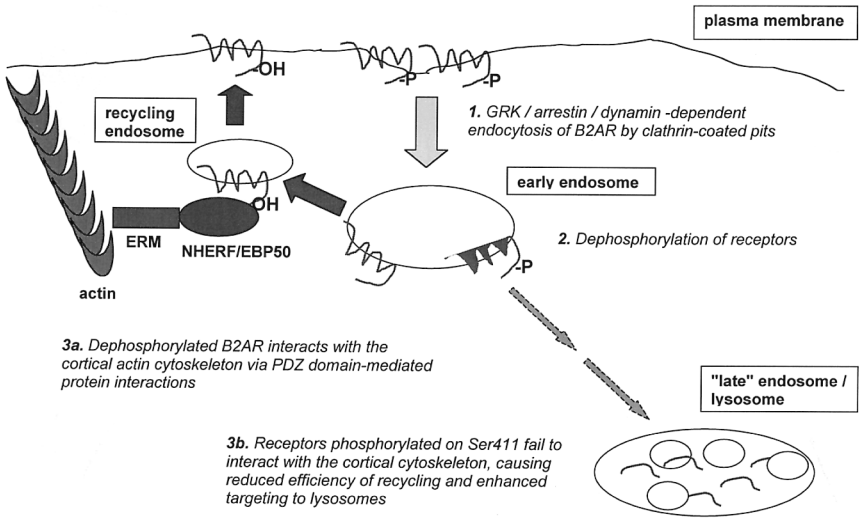

FIGURE 5.7. Model for endocytic sorting of the β_2-adrenergic receptor (B2AR) by phosphorylation-regulated association of receptors with the cortical actin cytoskeleton. B2ARs undergo rapid desensitization and endocytosis (step 1). If receptors are dephosphorylated (step 2), they are able to interact with a cytoskeleton-associated protein complex via a PDZ domain-mediated protein interaction with the carboxyl-terminal cytoplasmic domain of the receptor, which promotes rapid recycling of receptors to the plasma membrane (step 3a). If serine 411 in the receptor tail remains phosphorylated, this protein interaction does not occur, and recycling of receptors is inhibited. Receptors that fail to recycle can later be delivered to lysosomes, with down-regulation of receptors accomplished by proteolysis (step 3b).

family of proteins that interact with the B2AR via PDZ (PSD95/Discs large/ZO-1-homologous) domains and also associate with the cortical actin cytoskeleton (81). Overexpression of a mutant form of EBP50/NHERF that cannot interact with the cortical actin cytoskeleton, or chemical disruption of the actin cytoskeleton itself, also inhibits recycling of internalized B2ARs. These observations suggest that sorting of internalized B2ARs between a distinct recycling and degradative pathway can be mediated by a protein complex associated with the cortical actin cytoskeleton. Moreover, these studies suggest that phosphorylation of a specific serine residue (Ser411), a potential substrate for a subset of GRKs (82), can modulate the sorting of receptors by this mechanism (78). Thus, sorting of receptors after endocytosis, like the initial endocytosis of receptors from the plasma membrane, may be closely linked to the cycle of receptor phosphorylation and dephosphorylation involved in mediating desensitization and resensitization of signal transduction. A proposed model summarizing our current understanding of distinct stages of B2AR endocytosis and sorting is summarized in Fig. 5.7.

CONCLUSIONS AND FUTURE PERSPECTIVES

In this chapter, we have surveyed general processes of GPCR regulation and focused on selected mechanisms that are understood in molecular detail. A general principle emerging from this approach is that phosphorylation and membrane trafficking mechanisms are of fundamental importance to multiple processes of GPCR regulation. A second important principle is that distinct mechanisms of regulatory phosphorylation and membrane trafficking are closely interdependent, which leads to an appreciation of linked cycles coordinating the functional activation and regulation of receptors. An interesting corollary of this principle, suggested by certain recent studies of atypical antipsychotics and opiate analgesics (15), is that the linkage between specific mechanisms of receptor activation and regulation may be modified or disrupted by certain drugs. Therefore, it may be possible to target specific mechanisms of GPCR regulation, or the biochemical linkages between these mechanisms, to develop novel therapeutics that may influence the regulation of GPCRs in a manner quite different from that of classic agonists or antagonists.

We have restricted our focus to a subset of mechanisms that are, arguably, among the best established experimentally. It is important to note that these studies are in large part limited to model cell systems rather than native tissues. Nevertheless, the available data derived from transgenic and homologous recombination methodologies, and studies based on immunocytochemical localization of endogenously expressed GPCRs, suggest that phosphorylation (83, 84) and endocytosis of receptors do occur in native tissues in response to physiologic stimulation (85,86) and certain drugs (14,72). Thus, it is reasonable to propose that mechanisms elucidated with model cell systems may indeed be relevant to receptor regulation *in vivo.* However, it is not yet possible to predict precisely how specific mechanism(s) of regulation modulate the central nervous system under physiologic or pathophysiologic conditions, or how specific mechanisms of regulation may contribute to the *in vivo* effects of clinically important drugs. Thus, an important future goal is to examine the regulation of GPCRs in native cell types and tissues, and to determine how specific mechanisms of regulation contribute to physiologic and pathophysiologic states.

Finally, it is important to note that by restricting our scope to a limited subset of GPCR regulatory mechanisms, we have underrepresented the diversity of GPCR regulation and the high degree of specificity with which individual receptors are regulated in various cell types. For example, although we have discussed only endocytosis of GPCRs by clathrin-coated pits, substantial evidence indicates that other mechanism(s) also can mediate regulated endocytosis of receptors (37,87–89). Moreover, it is increasingly apparent that mechanisms of GPCR phosphorylation and endocytosis discussed in this chapter serve additional important functions in signal transduction, such as controlling the interaction of ''classic'' GPCR signaling pathways with mitogenic kinase cascades (see also Chapters 16 and 22) (90–92). Indeed, we anticipate that our present understanding of the array of GPCR signaling and regulatory mechanisms is at a relatively early stage of rapid development, a view consistent with the results of recent studies identifying an unexpected diversity of cellular proteins that interact with specific GPCRs (93). Future studies of these unexplored mechanisms of GPCR regulation may lead to important new insights relevant to neuropsychiatric disease and may identify exciting new targets for the development of novel therapeutic drugs.

REFERENCES

1. Clark RB. Receptor desensitization. *Adv Cyclic Nucl Prot Phos Res* 1986;20:151–209.
2. Ferguson SS, Zhang J, Barak LS, et al. Molecular mechanisms of G protein-coupled receptor desensitization and resensitization. *Life Sci* 1998;62:1561–1565.
3. Lefkowitz RJ, Pitcher J, Krueger K, et al. Mechanisms of beta-adrenergic receptor desensitization and resensitization. *Adv Pharmacol* 1998;42:416–420.
4. Carman CV, Benovic JL. G-protein-coupled receptors: turn-ons and turn-offs. *Curr Opin Neurobiol* 1998;8:335–344.
5. Lefkowitz RJ. G protein-coupled receptors and receptor kinases: from molecular biology to potential therapeutic applications. *Nat Biotechnol* 1996;14:283–286.
6. Roth BL, Willins DL, Kroeze WK. G protein-coupled receptor (GPCR) trafficking in the central nervous system: relevance for drugs of abuse. *Drug Alcohol Depend* 1998;51:73–85.
7. Perkins JP, Hausdorff WP, Lefkowitz RJ. Mechanisms of ligand-induced desensitization of beta-adrenergic receptors. In: Perkins

JP, ed. *The beta-adrenergic receptor.* Clifton, NJ: Humana Press, 1991:73–124.
8. Doss RC, Perkins JP, Harden TK. Recovery of beta-adrenergic receptors following long-term exposure of astrocytoma cells to catecholamine: role of protein synthesis. *J Biol Chem* 1981;256: 12281–12286.
9. Ng GY, Varghese G, Chung HT, et al. Resistance of the dopamine D2L receptor to desensitization accompanies the up-regulation of receptors on to the surface of Sf9 cells. *Endocrinology* 1997;138:4199–4206.
10. Zaki PA, Keith DE, Brine GA, et al. Ligand-induced changes in surface mu-opioid receptor number: relationship to G protein activation? *J Pharmacol Exp Ther* 2000;292:1127–1134.
11. Pfeiffer R, Kirsch J, Fahrenholz F. Agonist and antagonist-dependent internalization of the human vasopressin V2 receptor. *Exp Cell Res* 1998;244:327–339.
12. Willins DL, Berry SA, Alsayegh L, et al. Clozapine and other 5-hydroxytryptamine-2A receptor antagonists alter the subcellular distribution of 5-hydroxytryptamine-2A receptors *in vitro* and *in vivo. Neuroscience* 1999;91:599–606.
13. von Zastrow M, Keith DE, Zaki P, et al. Morphine and opioid peptide cause opposing effects on the endocytic trafficking of opioid receptors. *Regul Pept* 1994;54:315.
14. Keith DE, Anton B, Murray SR, et al. Mu-opioid receptor internalization: opiate drugs have differential effects on a conserved endocytic mechanism *in vitro* and in the mammalian brain. *Mol Pharmacol* 1998;53:377–384.
15. Roth BL, Willins DL. What's all the RAVE about receptor internalization? *Neuron* 1999;23:629–631.
16. Whistler JL, Chuang HH, Chu P, et al. Functional dissociation of mu opioid receptor signaling and endocytosis: implications for the biology of opiate tolerance and addiction. *Neuron* 1999; 23:737–746.
17. Chuang TT, Iacovelli L, Sallese M, et al. G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. *Trends Pharmacol Sci* 1996;17:416–421.
18. Bouvier M. Cross-talk between second messengers. *Ann N Y Acad Sci* 1990;594:120–129.
19. Krupnick JG, Benovic JL. The role of receptor kinases and arrestins in G protein-coupled receptor regulation. *Annu Rev Pharmacol Toxicol* 1998;38:289–319.
20. McDowell JH, Kuhn H. Light-induced phosphorylation of rhodopsin in cattle photoreceptor membranes: substrate activation and inactivation. *Biochemistry* 1977;16:4054–4060.
21. Bennett N, Sitaramayya A. Inactivation of photoexcited rhodopsin in retinal rods: the roles of rhodopsin kinase and 48-kDa protein (arrestin). *Biochemistry* 1988;27:1710–1715.
22. Sibley DR, Strasser RH, Caron MG, et al. Homologous desensitization of adenylate cyclase is associated with phosphorylation of the beta-adrenergic receptor. *J Biol Chem* 1985;260:3883–3886.
23. Benovic JL, Strasser RH, Caron MG, et al. Beta-adrenergic receptor kinase: identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor. *Proc Natl Acad Sci U S A* 1986;83:2797–2801.
24. Benovic JL, Stone WC, Huebner K, et al. cDNA cloning and chromosomal localization of the human beta-adrenergic receptor kinase. *FEBS Lett* 1991;283:122–126.
25. Lohse MJ, Benovic JL, Codina J, et al. Beta-arrestin: a protein that regulates beta-adrenergic receptor function. *Science* 1990; 248:1547–1550.
26. Benovic JL, Kuhn H, Weyand I, et al. Functional desensitization of the isolated beta-adrenergic receptor by the beta-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48-kDa protein). *Proc Natl Acad Sci U S A* 1987;84: 8879–8882.
27. Hausdorff WP, Lohse MJ, Bouvier M, et al. Two kinases mediate agonist-dependent phosphorylation and desensitization of the beta 2-adrenergic receptor. *Symp Soc Exp Biol* 1990;44:225–240.
28. Bouvier M, Hausdorff WP, De Blasi A, et al. Removal of phosphorylation sites from the beta 2-adrenergic receptor delays onset of agonist-promoted desensitization. *Nature* 1988;333:370–373.
29. Benovic JL, Bouvier M, Caron MG, et al. Regulation of adenylyl cyclase-coupled beta-adrenergic receptors. *Annu Rev Cell Biol* 1988;4:405–428.
30. Staehelin M, Simons P. Rapid and reversible disappearance of beta-adrenergic cell surface receptors. *EMBO J* 1982;1:187–190.
31. Toews ML, Perkins JP. Agonist-induced changes in beta-adrenergic receptors on intact cells. *J Biol Chem* 1984;259:2227–2235.
32. von Zastrow M, Kobilka BK. Ligand-regulated internalization and recycling of human beta 2-adrenergic receptors between the plasma membrane and endosomes containing transferrin receptors. *J Biol Chem* 1992;267:3530–3538.
33. Kurz JB, Perkins JP. Isoproterenol-initiated beta-adrenergic receptor diacytosis in cultured cells. *Mol Pharmacol* 1992;41: 375–381.
34. von Zastrow M, Kobilka BK. Antagonist-dependent and -independent steps in the mechanism of adrenergic receptor internalization. *J Biol Chem* 1994;269:18448–18452.
35. van der Bliek AM, Redelmeier TE, Damke H, et al. Mutations in human dynamin block an intermediate stage in coated vesicle formation. *J Cell Biol* 1993;122:553–563.
36. Herskovits JS, Burgess CC, Obar RA, et al. Effects of mutant rat dynamin on endocytosis. *J Cell Biol* 1993;122:565–578.
37. Zhang J, Ferguson S, Barak LS, et al. Dynamin and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. *J Biol Chem* 1996;271:18302–18305.
38. Cao TC, Mays RW, von Zastrow M. Regulated endocytosis of G protein-coupled receptors by a biochemically and functionally distinct subpopulation of clathrin-coated pits. *J Biol Chem* 1998; 273:24592–24602.
39. Tsuga H, Kameyama K, Haga T, et al. Sequestration of muscarinic acetylcholine receptor m2 subtypes. Facilitation by G protein-coupled receptor kinase (GRK2) and attenuation by a dominant-negative mutant of GRK2. *J Biol Chem* 1994;269: 32522–32527.
40. Goodman OJ, Krupnick JG, Santini F, et al. Beta-arrestin acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor. *Nature* 1996;383:447–450.
41. Goodman OB Jr, Krupnick JG, Santini F, et al. Role of arrestins in G-protein-coupled receptor endocytosis. *Adv Pharmacol* 1998; 42:429–433.
42. Krupnick JG, Santini F, Gagnon AW, et al. Modulation of the arrestin–clathrin interaction in cells. Characterization of beta-arrestin dominant-negative mutants. *J Biol Chem* 1997;272: 32507–32512.
43. Krupnick JG, Goodman OJ, Keen JH, et al. Arrestin/clathrin interaction. Localization of the clathrin-binding domain of non-visual arrestins to the carboxy terminus. *J Biol Chem* 1997;272: 15011–15016.
44. Wedegaertner PB, Bourne HR, von Zastrow M. Activation-induced subcellular redistribution of Gs alpha. *Mol Biol Cell* 1996;7:1225–1233.
45. Ransnas LA, Jasper JR, Leiber D, et al. Beta-adrenergic-receptor-mediated dissociation and membrane release of the Gs protein in S49 lymphoma-cell membranes. Dependence on Mg^{2+} and GTP. *Biochem J* 1992;281:519–524.
46. Pak Y, Kouvelas A, Scheideler MA, et al. Agonist-induced functional desensitization of the mu-opioid receptor is mediated by loss of membrane receptors rather than uncoupling from G protein. *Mol Pharmacol* 1996;50:1214–1222.

47. von Zastrow M, Link R, Daunt D, et al. Subtype-specific differences in the intracellular sorting of G protein-coupled receptors. *J Biol Chem* 1993;268:763–766.
48. Daunt DA, Hurt C, Hein L, et al. Subtype-specific intracellular trafficking of alpha2-adrenergic receptors. *Mol Pharmacol* 1997; 51:711–720.
49. Pippig S, Andexinger S, Daniel K, et al. Overexpression of beta-arrestin and beta-adrenergic receptor kinase augment desensitization of beta 2-adrenergic receptors. *J Biol Chem* 1993;268: 3201–3208.
50. Yu SS, Lefkowitz RJ, Hausdorff WP. Beta-adrenergic receptor sequestration. A potential mechanism of receptor resensitization. *J Biol Chem* 1993;268:337–341.
51. Pitcher JA, Payne ES, Csortos C, et al. The G-protein-coupled receptor phosphatase: a protein phosphatase type 2A with a distinct subcellular distribution and substrate specificity. *Proc Natl Acad Sci U S A* 1995;92:8343–8347.
52. Pippig S, Andexinger S, Lohse MJ. Sequestration and recycling of beta 2-adrenergic receptors permit receptor resensitization. *Mol Pharmacol* 1995;47:666–676.
53. Koenig JA, Edwardson JM. Endocytosis and recycling of G protein-coupled receptors. *Trends Pharmacol Sci* 1997;18:276–287.
54. Law P-Y, Hom DS, Loh HH. Down-regulation of opiate receptor in neuroblastoma x glioma NG108-15 hybrid cells: chloroquine promotes accumulation of tritiated enkephalin in the lysosomes. *J Biol Chem* 1984;259:4096–4104.
55. Ko JL, Arvidsson U, Williams FG, et al. Visualization of time-dependent redistribution of delta-opioid receptors in neuronal cells during prolonged agonist exposure. *Brain Res Mol Brain Res* 1999;69:171–185.
56. Kojro E, Fahrenholz F. Ligand-induced cleavage of the V2 vasopressin receptor by a plasma membrane metalloproteinase. *J Biol Chem* 1995;270:6476–6481.
57. Jockers R, Angers S, Da Silva A, et al. Beta(2)-adrenergic receptor down-regulation. Evidence for a pathway that does not require endocytosis. *J Biol Chem* 1999;274:28900-28908.
58. Bochtler M, Ditzel L, Groll M, et al. The proteasome. *Annu Rev Biophys Biomol Struct* 1999;28:295–317.
59. Strous GJ, van Kerkhof P, Govers R, et al. The ubiquitin conjugation system is required for ligand-induced endocytosis and degradation of the growth hormone receptor. *EMBO J* 1996;15: 3806–3812.
60. Hicke L. Gettin' down with ubiquitin: turning off cell-surface receptors, transporters and channels. *Trends Cell Biol* 1999;9: 107–112.
61. Levkowitz G, Waterman H, Ettenberg SA, et al. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol Cell* 1999;4: 1029–1040.
62. Gruenberg J, Maxfield FR. Membrane transport in the endocytic pathway. *Curr Opin Cell Biol* 1995;7:552–563.
63. Law P-Y, Hom DS, Loh HH. Loss of opiate receptor activity in neuroblastoma x glioma NG108-15 cells after chronic etorphine treatment: a multiple step process. *Mol Pharmacol* 1982;72:1–4.
64. Campbell PT, Hnatowitch M, O'Dowd BF, et al. Mutational analysis of adrenergic receptor sequestration. *Mol Pharmacol* 1991;39:192–198.
65. Bouvier M, Collins S, O'Dowd BF, et al. Two distinct pathways for cAMP-mediated down-regulation of the beta 2-adrenergic receptor. Phosphorylation of the receptor and regulation of its mRNA level. *J Biol Chem* 1989;264:16786–16792.
66. Hausdorff WP, Bouvier M, O'Dowd BF, et al. Phosphorylation sites on two domains of the beta 2-adrenergic receptor are involved in distinct pathways of receptor desensitization. *J Biol Chem* 1989;264:12657–12665.
67. Hausdorff WP, Campbell PT, Ostrowski J, et al. A small region of the beta-adrenergic receptor is selectively involved in its rapid regulation. *Proc Natl Acad Sci U S A* 1991;88:2979–2983.
68. Heck DA, Bylund DB. Differential down-regulation of alpha-2 adrenergic receptor subtypes. *Life Sci* 1998;62:1467–1472.
69. von Zastrow M, Keith DJ, Evans CJ. Agonist-induced state of the delta-opioid receptor that discriminates between opioid peptides and opiate alkaloids. *Mol Pharmacol* 1993;44:166–172.
70. Arden JR, Segredo V, Wang Z, et al. Phosphorylation and agonist-specific intracellular trafficking of an epitope-tagged mu-opioid receptor expressed in HEK 293 cells. *J Neurochem* 1995;65: 1636–1645.
71. Keith DE, Murray SR, Zaki PA, et al. Morphine activates opioid receptors without causing their rapid internalization. *J Biol Chem* 1996;271:19021–19024.
72. Sternini C, Spann M, Anton B, et al. Agonist-selective endocytosis of mu opioid receptor by neurons *in vivo. Proc Natl Acad Sci U S A* 1996;93:9241–9246.
73. Trejo J, Coughlin SR. The cytoplasmic tails of protease-activated receptor-1 and substance P receptor specify sorting to lysosomes versus recycling. *J Biol Chem* 1999;274:2216–2224.
74. Mellman I. Endocytosis and molecular sorting. *Annu Rev Cell Dev Biol* 1996;12:575–625.
75. Gagnon AW, Kallal L, Benovic JL. Role of clathrin-mediated endocytosis in agonist-induced down-regulation of the beta2-adrenergic receptor. *J Biol Chem* 1998;273:6976–6981.
76. Chu P, Murray S, Lissin D, et al. Delta and kappa opioid receptors are differentially regulated by dynamin-dependent endocytosis when activated by the same alkaloid agonist. *J Biol Chem* 1997;272:27124–27130.
77. Tsao PI, von Zastrow M. Type-specific sorting of G protein-coupled receptors after endocytosis. *J Biol Chem* 2000;275: 11130–11140.
78. Cao TT, Deacon HW, Reczek D, et al. A kinase-regulated PDZ-domain interaction controls endocytic sorting of the beta2-adrenergic receptor. *Nature* 1999;401:286–290.
79. Hall RA, Premont RT, Chow CW, et al. The beta2-adrenergic receptor interacts with the Na^+/H^+-exchanger regulatory factor to control Na^+/H^+ exchange. [Nature] 1998;392:626–630.
80. Hall RA, Ostedgaard LS, Premont RT, et al. A C-terminal motif found in the beta2-adrenergic receptor, P2Y1 receptor and cystic fibrosis transmembrane conductance regulator determines binding to the Na^+/H^+ exchanger regulatory factor family of PDZ proteins. *Proc Natl Acad Sci U S A* 1998;95:8496–8501.
81. Reczek D, Berryman M, Bretscher A. Identification of EBP50: a PDZ-containing phosphoprotein that associates with members of the ezrin–radixin–moesin family. *J Cell Biol* 1997;139: 169–179.
82. Fredericks ZL, Pitcher JA, Lefkowitz RJ. Identification of the G protein-coupled receptor kinase phosphorylation sites in the human beta2-adrenergic receptor. *J Biol Chem* 1996;271: 13796–13803.
83. Gainetdinov RR, Bohn LM, Walker JK, et al. Muscarinic supersensitivity and impaired receptor desensitization in G protein-coupled receptor kinase 5-deficient mice. *Neuron* 1999;24: 1029–1036.
84. Eckhart AD, Duncan SJ, Penn RB, et al. Hybrid transgenic mice reveal *in vivo* specificity of G protein-coupled receptor kinases in the heart. *Circ Res* 2000;86:43–50.
85. Mantyh PW, DeMaster E, Malhotra A, et al. Receptor endocytosis and dendrite reshaping in spinal neurons after somatosensory stimulation. *Science* 1995;268:1629–1632.
86. Mantyh PW, Allen CJ, Ghilardi JR, et al. Rapid endocytosis of

a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum *in vivo. Proc Natl Acad Sci U S A* 1995;92:2622–2626.
87. Pals-Rylaarsdam R, Gurevich VV, Lee KB, et al. Internalization of the m2 muscarinic acetylcholine receptor. Arrestin-independent and -dependent pathways. *J Biol Chem* 1997;272: 23682–23689.
88. Lee KB, Pals RR, Benovic JL, et al. Arrestin-independent internalization of the m1, m3, and m4 subtypes of muscarinic cholinergic receptors. *J Biol Chem* 1998;273:12967–12972.
89. Vickery RG, von Zastrow M. Distinct dynamin-dependent and -independent mechanisms target structurally homologous dopamine receptors to different endocytic membranes. *J Cell Biol* 1999;144:31–43.
90. Luttrell LM, Ferguson SS, Daaka Y, et al. Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science* 1999;283:655–661.
91. DeFea KA, Zalevsky J, Thoma MS, et al. Beta-arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. *J Cell Biol* 2000;148: 1267–1281.
92. Miller WE, Maudsley S, Ahn S, et al. Beta-arrestin1 interacts with the catalytic domain of the tyrosine kinase c-SRC. Role of beta-arrestin 1-dependent targeting of c-SRC in receptor endocytosis. *J Biol Chem* 2000;275:11312–11319.
93. Hall RA, Premont RT, Lefkowitz RJ. Heptahelical receptor signaling: beyond the G protein paradigm. *J Cell Biol* 1999;145: 927–932.

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