β-Arrestins: Multifunctional Cellular Mediators

Initially thought to play a role only in G-protein-coupled receptor desensitization, β-arrestins are ascribed with new roles such as scaffolding and signaling proteins by their own right. This review explores the many functions of β-arrestins, with an emphasis on their recently identified role as regulators of receptor signaling.

Arrestin, also termed 48-kDa protein or S antigen, was initially identified in the mid-1980s as a cytosolic protein that arrest s cGMP phosphodiesterase activation in retinal rod disk membranes, serving as a co-factor in dampening the responsiveness of the light receptor rhodopsin to photoactivation by rhodopsin kinase (66, 67). The observation that desensitization of another member of the G-protein-coupled receptor (GPCR) family, β-adrenergic receptor (βAR), by its purified β-adrenergic receptor kinase (βARK) also required the presence of arrestin (4), was the gateway to the extension of its role to a general theme in GPCR signaling. Indeed, multiple studies in a host of receptors confirm this classical function of arrestin (11, 31).

In vertebrates, four members of the arrestin family have been cloned. These are the visual arrestins (arrestins 1 and 4), where expression is limited to retinal rods and cones, and arrestins 2 and 3 (also called β-arrestin 1 and 2), which are ubiquitously expressed in mammalian tissue (25). Structural information of unbound visual and nonvisual arrestin reveals a basic unit comprised of two domains of antiparallel β-sheets connected through a hinge region and one short α-helix at the back of the amino-terminal tail (17). The point of interaction of arrestin with the receptor is a cationic amphipathic helix that may function as a reversible membrane anchor to facilitate the formation of a high-affinity complex between β-arrestin and the ligand-activated GPCR (18, 60). The transition from the basal inactive conformation to the biologically active one was shown to require conformational changes occurring within the protein itself (42, 69). Arrestins also contain specific interaction sites with multiple signaling and scaffolding molecules, such as phospholipids and β-adap tin (36, 37), thus providing it with the capacity of regulating many different cellular functions such as membrane, cytosolic, and nuclear-associated signaling and trafficking. Further examination of the higher order structures of arrestin reveal the existence of an equilibrium between monomeric forms of the protein, which represent the active form capable of binding to the activated receptor, and a tetramer that is thought to function as a storage form to supply active monomer when needed to quench receptor signaling (19, 23). Oligomerization of arrestins also affects their subcellular localization. Whereas the tetrameric form restricts the protein mainly to the cytosol, the monomers increase its nuclear localization (36).

Role of β-Arrestin in Receptor Desensitization

Loss of responsiveness of GPCRs to an ongoing stimulus or desensitization is a process that has been studied extensively (see Refs. 28, 34, 71). Briefly, the classical paradigm for desensitization involves the dual step of receptor phosphorylation by second messenger-stimulated protein kinases (i.e., PKA or PKC, termed heterologous desensitization) or specific G-protein-coupled receptor kinases (GRKs, termed homologous desensitization) and subsequent binding of arrestin to sterically interdict further coupling between the receptor and the G protein. Together, these two actions regulate functional coupling of receptors to effector molecules and control the subcellular localization of the receptor during the process of agonist-induced homologous desensitization (4, 59) (FIGURE 1).

A more recently discovered β-arrestin-mediated regulatory mechanism for desensitization involves the degradation of second messenger, such as adenyl cyclase-generated cAMP, by scaffolding phosphodiesterases (PDEs) to the vicinity of the effector (46). Whereas these two desensitization processes were thought in the past to be independent of one another, recruitment of both β-arrestin and PDE to activated βARs showed very similar kinetics, suggesting that β-arrestins may serve as shuttles for the translocation of PDE to the activated receptor (46). This observation was strengthened by the fact that cells lacking β-arrestin expression were unable to recruit PDE following βAR stimulation, a function that was rescued by the introduction of exogenous β-arrestin to cells (46). β-Arrestin-mediated recruitment of PDE to activated βARs was shown in a subsequent study performed in cardiac myocytes to promote the switching from Gs to Gi coupling, thus shifting the receptor toward a pathway that further limits cAMP production (3).

Similar to its role in shuttling PDE to the Gs-/Gi-coupled βAR, β-arrestin was recently found to reduce the levels of Gq-/PKC-generated second messenger diacylglycerol (DAG) following agonist stimulation of...
the M1 cholinergic receptor (41). Coordinating the translocation of diacylglycerol kinase (DGK) to the ligand-stimulated β-arrestin receptor promotes the degradation of DAG to phosphatidic acid (41). Importantly, expression of β-arrestin mutants that bind DGK but cannot bind to activated receptors generated significantly lower amounts of DAG, suggesting that scaffolding of DGK by β-arrestin is able to desensitize signaling independent of steric hindrance. Together, these studies support two new general roles for arrestin in desensitization: 1) limiting second messenger generation and 2) enhancing the rate of second messenger degradation.

Receptor Trafficking: Internalization, Recycling, and Degradation

The exposure of agonist results in trafficking of GPCRs into intracellular compartments in a process of sequestration or internalization. Internalization was initially identified as a critical step in resensitization of desensitized receptors (59) but more recently was shown to initiate cellular signaling such as mitogenic pathways (8, 32, 43). A central role for β-arrestin in the internalization process was discovered by showing that overexpression of β-arrestin rescues internalization-deficient βAR mutants impaired in their sequestration ability and conversely that βAR internalization can be inhibited with β-arrestin-defective mutants (12).

The mechanism by which β-arrestin mediates receptor internalization is via its ability to interact with proteins of the clathrin-coated pit (CCP) machinery. It is now understood that β-arrestin functions as an adaptor molecule that binds directly to clathrin via the adaptor protein AP-2 (16, 27). AP-2 recruitment to the plasma membrane is facilitated by a direct agonist-dependent interaction between GRK2 and PI3 kinase in the cytosol, followed by rapid translocation of both enzymes to the plasma membrane where they interact with agonist-activated receptors (38, 40). The generation of 3,4,5-phosphatidylinositols at the membrane by PI3 kinase enhances the recruitment of AP-2, thus promoting endocytosis (38, 40). Interestingly, the internalization process also requires protein kinase activity of PI3 kinase, which acts to phosphorylate cytoskeletal tropomyosin, allowing for actin polymerization (39).

Complex formation between the receptor and the components of the sequestration machinery depends on the phosphorylation state of β-arrestin. In the unstimulated state, β-arrestin is constitutively phosphorylated by the mitogen-activated protein kinase (MAPK) ERK1/2, thus reducing its capacity to interact with clathrin. On agonist stimulation of βARs, cytoplasmic β-arrestin is recruited to the plasma membrane, where it is rapidly dephosphorylated, an action that promotes its binding to actin and subsequent endocytosis (30) (FIGURE 1).

The mechanism by which β-arrestin regulates GPCR trafficking is complex, since arrestins display varying binding affinity to different GPCRs. Assays of arrestin-receptor interactions in live cells showed different localization patterns of arrestins: whereas visual arrestin and β-arrestin 1 can be found in both the cytoplasm and the nucleus, β-arrestin 2 is limited to...
the cytoplasm (44, 53). This observation provides the basis for classifying GPCRs according to the type of interaction they have with arrestin. The first group, termed class A receptors (e.g., βARs, μ opioid receptors, and D1 dopamine receptors), bind β-arrestin 2 with a greater affinity than β-arrestin 1 (70). The interaction between class A receptors and β-arrestin is transient and is lost during internalization. Receptors in the second group, termed class B receptors (e.g., angiotensin II type 1A receptor, neurotensin receptor 1, and vasopressin V2 receptor), bind β-arrestin 1 and 2 with equal affinity, and the interaction remains intact during internalization. Interestingly, switching the carboxy tail of a class A receptor to a class B receptor reverses the receptor affinity to the different β-arrestins (44, 70). These data suggest that GPCR signaling is differentially regulated, depending on the cellular complement of arrestin isoforms and their ability to interact with other cellular proteins (44).

Once internalized into intracellular compartments, receptors are destined to either recycle back to the plasma membrane or, alternatively, become targeted for postendocytic degradation. One of the cellular processes that determines the fate of the receptor is ubiquitination—the adding of multiple ubiquitin molecules to lysine residues of the substrate protein, an action that marks it for degradation by the proteosome (20). Indeed, several GPCRs such as rhodopsin, βAR and μ opioid receptors undergo agonist-mediated ubiquitination (6, 45, 58). However, although ubiquitination of the β2AR is necessary for receptor degradation, concomitantly occurring ubiquitination of β-arrestin on agonist stimulation is also required for βAR internalization. The kinetics of βAR and β-arrestin ubiquitination differ in that compared to the receptor, ubiquitination of β-arrestin is more rapid and transient, corresponding to the time frame of its association with the class A receptor (58). Chimeric receptors, constructed by switching the cytoplasmic tails between two receptor classes (class A βAR and class B V2 vasopressin receptors), reverses the pattern of both β-arrestin trafficking and ubiquitination of the respective receptor. Furthermore, the use of a permanently ubiquitinated β-arrestin mutant, which prevents dissociation from the βAR, transforms the internalization pattern from a class A-type into a class B-type receptor (57). Together, these data suggest that the ubiquitination status of β-arrestin determines the stability of the receptor-β-arrestin complex in addition to its trafficking pattern (29, 57).

Other than its role in GPCR ubiquitination, β-arrestin was recently shown to mediate the regulation of additional receptor types (7, 14, 68). One of those is the insulin-like growth factor-1 receptor (IGF-1R), which mediates several crucial functions in cell growth and differentiation. For the IGF-1R, β-arrestin serves as an adaptor, binding the E3 ubiquitin ligase Mdm2 to the IGF-1R and leading to its proteasome-dependent degradation (14). Stimulation of IGF-1R also leads to ubiquitination of β-arrestin 1, an event that regulates ERK1/2 activation and cell cycle progression (15). Competitive binding of β-arrestin to Mdm2 also displaces the insulin receptor substrate 1 (IRS-1) from Mdm2, thereby decreasing its rate of ubiquitination and degradation and increasing insulin signaling (61).

“A considerable amount of in vitro data now indicates that activation of a GPCR can promote G-protein-independent/β-arrestin-mediated signaling . . .

β-Arrestins as Transducers of Receptor Signaling

The classical paradigm for signaling of GPCRs proposes a three-step model whereby an agonist-activated receptor couples to a heterotrimeric G protein, which leads to the dissociation of the heterotrimer and to subsequent activation of effector molecules. However, more recent data suggests that “G-protein-coupled” receptors are capable of activating signaling pathways independent of G-protein activation (18, 54, 64). This section explores the function of β-arrestin as a transducer of cellular signaling.

Apart from their long-recognized roles in intermediary metabolism, GPCRs function as important regulators of cell proliferation and differentiation, mainly through the activation of mitogenic pathways, among which the ERK1/2 MAPK cascade is the best studied. Activation of these pathways by GPCRs has led to the discovery of a new role for β-arrestins as adaptor molecules that promote the formation of multi-protein signaling complexes with proteins such as ERK and receptor and nonreceptor tyrosine kinases (32, 33, 35). This important phenomenon was observed for the signaling of angiotensin II type 1 receptor (AT1R). Although angiotensin II (AngII) stimulation of an AT1R with a mutation in the DRY motif of its second intracellular loop did not yield any G protein-dependent signaling, β-arrestin recruitment and ERK activation remained intact. The same result was obtained by stimulation of a wild-type AT1R with the AngII analog [Sar(1), Ile(4), Ile(8)]-angiotensin II (SII) (21, 22, 54, 64). Furthermore, depletion of cellular β-arrestin 2 by small interfering RNA (siRNA) or the use of phosphorylation-deficient AT1R mutants blocked the angiotensin-stimulated ERK response, suggesting the existence of a signaling pathway that is G protein independent but β-arrestin dependent (64). Kinetics of the two pathways are that G protein-dependent activation is rapid, is transient (peaking at 2 min and subsiding within minutes), and leads to nuclear translocation of ERK, whereas β arrestin-dependent activation is slower (peaking at 5-10 min and subsiding over hours) and confines ERK to the
Role of GRKs

The interaction of β-arrestin with an activated receptor is mediated by the small family of GRKs comprised of seven members (GRK1–7). GRK1 and 7 are exclusively expressed in the retina, whereas the rest are ubiquitously expressed in mammalian tissues. In the GRK family, GRK2 and 3 interact with Gβγ subunits and reside in the cytoplasm, whereas GRK4, 5, and 6 are membrane bound (48). It appears that there is a specific subset of GRKs that mediate β-arrestin-dependent signaling for both class A (β2ARs) and class B (AT1R) receptors (24, 56, 63). For the β2AR, GRK5 and 6, but not GRK2 and 3, induce receptor phosphorylation that leads to agonist-stimulated ERK activation in the absence of G-protein activation (56). Similarly, siRNA silencing of GRK2, 3, 5, and 6 during activation of wild-type AT1Rs showed that, while lowering the expression of GRK2 and 3, increases in β-arrestin-mediated signaling attenuation of the expression of GRK5 or 6 abolishes β-arrestin-mediated activation of ERK (24).

One of the immediate implications that arises from the above data is that receptor phosphorylation by the different GRKs, together with β-arrestin binding, may be a critical factor in directing receptor signaling toward G-protein-dependent or -independent pathways. This was recently shown for downstream signaling following activation of the β1-adrenergic receptor (43). β1ARs have the capacity to internalize via two distinct pathways, namely CCPs or caveolae, each directing the receptor to a different microdomain (2, 5). The determining factor as to which pathway is selected is whether the activated receptor is phosphorylated by PKA or by GRKs. Thus mutant β1ARs that lack the ability to undergo PKA phosphorylation but are able to undergo GRK phosphorylation internalize via CCP in a β-arrestin-dependent manner. Conversely, receptors lacking GRK phosphorylation sites are sequestered via caveolae in a β-arrestin-independent manner (51). Interestingly, β1ARs can signal through a β-arrestin-dependent pathway through a process known as EGF receptor transactivation (43). GPCR-mediated transactivation of receptor tyrosine kinases such as the EGF receptor has been the subject of rigorous investigation in the past decade (for reviews, see Refs. 47, 72). The current paradigm of GPCR-mediated transactivation involves a series of partially defined steps that leads to matrix metalloprotease-mediated cleavage and shedding of heparin-binding EGF (HB-EGF) ligand to activate the EGF receptor (10, 49, 55). For the β1AR, agonist-induced transactivation of the EGFR is not only dependent on GRK-mediated phosphorylation of the β1AR but also requires both β-arrestin 1 and 2 (43). Furthermore, similar to the findings regarding ERK activation by β1ARs (24), β-arrestin-mediated transactivation is dependent on selective phosphorylation of the receptor by GRK5 or 6 (FIGURE 1).

Physiological Consequences of β-Arrestin-Dependent Signaling

A considerable amount of in vitro data now indicates that activation of a GPCR can promote G-protein-independent/β-arrestin-mediated signaling, suggesting that receptors can assume distinct conformations, each responsible for a distinct pathway that can be potentially stimulated by unique ligands.

β-Arrestin Signaling in the Heart

An important example of β-arrestin-mediated signaling in vivo was recently identified from the study of β1AR-induced transactivation of the EGFR receptor in the heart. Here, knockout mice individually lacking the genes encoding β-arrestin 2, GRK5, or GRK6 were used to demonstrate that stimulation of β1ARs induced β-arrestin/GRK- or 6-mediated transactivation of the EGFR receptor (43). The physiological relevance of this pathway was demonstrated using transgenic mice overexpressing wild-type β1ARs or mutant β1ARs lacking either GRK or PKA phosphorylation sites. Under conditions of chronic catecholamine stimulation, mice overexpressing mutant β1ARs that lack GRK phosphorylation sites and unable to induce β-arrestin mediated EGFR transactivation showed significant deterioration in cardiac function (43). These findings suggest that...
cardiac βAR-β-arrestin-mediated cascades confer cardioprotection in pathways that are independent of classic G-protein-coupled signaling.

Concluding Remarks

The roles for β-arrestin have evolved in the past two decades from a molecule involved merely in interdicting agonist-mediated receptor G-protein coupling to one with novel mechanisms of action in desensitization, trafficking cellular signaling. The many functions of β-arrestin position it at the junction of diverging signaling cellular pathways, providing tremendous potential therapeutic implications. The challenge faced by novel drug discovery will be to identify the desirable signaling pathway in a given GPCR-related disease (i.e., G-protein-dependent, β-arrestin-dependent) and to design "perfectly biased" ligands that can direct signaling down that pathway. Further understanding of the mechanisms that underlie alternative signaling pathways by GPCRs is likely to lead to the development of novel GPCR-targeted drugs with dual function of blocking one pathway while activating another. ■

References


Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, Ullrich A. EGF receptor transactivation by G-coupled-protein receptors requires met-


Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor, 1998.

Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor, 1998.

Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor, 1998.

Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor, 1998.

Sibley DR, Strasser RH, Benovic JL, Daniel K, Lefkowitz RJ. Phosphorylation/dephosphorylation of the beta-adrenergic receptor regulates its func-
tional coupling to adenylyl cyclase and subcellu-

Sutton RB, Vashnevskiy SA, Robert J, Hanson SM, Raman D, Knox BE, Kano M, Navarro J, Gurevich VV. Crystal structure of cone arrestin at 2.4A: evo-


