Topical Review

G-protein coupled receptor kinases as modulators of G-protein signalling

Moritz Bünemann and M. Marlene Hosey

Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, 303 East Chicago Avenue S215, Chicago, IL 60611, USA

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G-protein coupled receptors (GPCRs) comprise one of the largest classes of signalling molecules. A wide diversity of activating ligands induce the active conformation of GPCRs and lead to signalling via heterotrimeric G-proteins and downstream effectors. In addition, a complex series of reactions participate in the 'turn-off' of GPCRs in both physiological and pharmacological settings. Some key players in the inactivation or 'desensitization' of GPCRs have been identified, whereas others remain the target of ongoing studies. G-protein coupled receptor kinases (GRKs) specifically phosphorylate activated GPCRs and initiate homologous desensitization. Uncoupling proteins, such as members of the arrestin family, bind to the phosphorylated and activated GPCRs and cause desensitization by precluding further interactions of the GPCRs and G-proteins. Adaptor proteins, including arrestins, and endocytic machinery participate in the internalization of GPCRs away from their normal signalling milieu. In this review we discuss the roles of these regulatory molecules as modulators of GPCR signalling.

A broad spectrum of extracellular signals, such as hormones, neurotransmitters, chemokines, odorants and light, are detected by members of the family of G-protein coupled receptors (GPCRs). More than a thousand members of this receptor family are predicted to exist. All GPCRs identified to date share the typical structural motif of seven membrane-spanning helices, and all convert extracellular signals into intracellular signals by activating heterotrimeric G-proteins. In addition to the ligands that activate the GPCRs, there are a number of other regulatory factors that influence the activation and deactivation of GPCR signalling, and hence play important roles in the specificity and spatio-temporal patterns of physiological responses to extracellular signals.

In the simplest model, GPCRs can be viewed as proteins that can exist in an inactive or active state (Gether & Kobilka, 1998). While increasing evidence is emerging that some GPCRs exhibit constitutive activity in the absence of ligand (Leurs *et al.* 1998), under normal conditions, and in the absence of their respective activating ligands, the inactive conformation is favoured (Gether & Kobilka, 1998). Upon activation by ligands, GPCRs are converted into the active conformation and are able to complex with and activate heterotrimeric G-proteins. The heterotrimeric G-proteins are composed of three subunits: the α-subunit which carries the guanine-nucleotide binding site, and the β- and γ-subunits which form a tightly bound dimer. Inactive G-proteins are heterotrimers composed of a GDP-bound α-subunit associated with the G$\beta\gamma$-dimer (Fig. 1). The activated GPCRs function as GDP/GTP exchange factors and promote the release of GDP and the binding of GTP to the α-subunits. This leads to dissociation of the α-subunit and the G$\beta\gamma$-dimer (Fig. 1). Both GTP–Gα and G$\beta\gamma$ can interact with a variety of effector systems in order to modulate cellular signalling pathways (Hamm, 1998). The deactivation of GPCR signalling occurs at several levels. Importantly, the G-protein Gα-subunit hydrolyses GTP to GDP and in turn reassociates with G$\beta\gamma$ to form the inactive heterotrimer (Fig. 1). In addition, ligand dissociation from the GPCRs converts the receptors back to their inactive state.

Several mechanisms exist to regulate the length and strength of GPCR signals. In many cases a time-dependent decrease of the cellular response to the external signal occurs despite the continued presence of the signalling ligand. This attenuation of signalling is known as desensitization, and is important in physiological and pharmacological settings. In principal, desensitization of a G-protein-mediated signal can be achieved due to attenuation of signalling at the level of the receptor, the G-protein or the effector system, and examples of each are known. Indeed, there has been much interest recently in proteins termed the regulators of G-protein signalling (RGS proteins) (Berman & Gilman, 1998) that accelerate the hydrolysis of Gα-bound GTP and promote the deactivation of G-proteins (Fig. 1). RGS proteins may be important for the turn-off of many physiological responses; however, our understanding of their roles is only

now being elucidated. The most extensively studied mechanism of desensitization is that occurring at the level of the GPCRs and is the main focus of this review.

Desensitization of G-protein coupled receptors

Many GPCRs have been shown to desensitize during the course of exposure to agonist (Krupnick & Benovic, 1998; Pitcher *et al.* 1998*a*; Bünemann *et al.* 1999). The time course and extent of receptor desensitization vary depending on the type of receptor and cellular background. Two general forms of desensitization have been described. Homologous desensitization refers to the situation whereby only the activated GPCRs desensitize, while heterologous desensitization refers to the situation whereby activation of one GPCR leads to the desensitization of responses initiated by another, heterologous GPCR. Multiple mechanisms of receptor desensitization exist, and not all are understood. However, agonist-dependent phosphorylation of the GPCRs appears to play an important role in initiating the desensitization of many GPCRs (Freedman & Lefkowitz, 1996; Pitcher *et al.* 1998*a*). While classical second-messenger-activated protein kinases may participate in the desensitization of various types of GPCRs, a unique family of protein kinases known as the G-protein coupled receptor kinases (GRKs) has been proposed to be responsible for agonist-dependent phosphorylation of GPCRs and to play a key role in initiating homologous desensitization.

A GRK-dependent pathway of desensitization was elucidated first in studies of the visual receptor rhodopsin (Kuhn, 1974; Weller *et al.* 1975; Zhao *et al.* 1995). Subsequently, a similar pathway was identified in studies of desensitization of β_2-adrenergic receptors (β_2AR). Because the β_2AR has been the most extensively studied GPCR in terms of desensitization (Krupnick & Benovic, 1998; Lefkowitz, 1998; Pitcher *et al.* 1998*a*), the pathway that leads to desensitization of the β_2AR has been viewed as a paradigm for other GPCRs. Indeed, in the past several years, other GPCRs have been shown to be regulated by GRKs in a manner similar to β_2ARs (Krupnick & Benovic, 1998; Pitcher *et al.* 1998*a*). However, it is also becoming increasingly clear that there are many variations in the molecular events associated with desensitization of GPCRs and that the model developed for the β_2AR does not apply in all respects to all GPCRs. In this review, we will focus on the roles of GRKs and the mechanisms of desensitization that have been elucidated for GPCRs other than the β_2AR, as many excellent reviews already exist that describe the events associated with the desensitization of the β_2AR (Freedman & Lefkowitz, 1996; Lefkowitz, 1998; Pitcher *et al.* 1998*a*). The general model developed to describe GRK-dependent desensitization is shown in Fig. 2. After activation of GPCRs by their respective agonists, GRKs specifically phosphorylate the agonist-activated receptors, leaving the non-activated receptors unaffected. This specificiy of GRKs

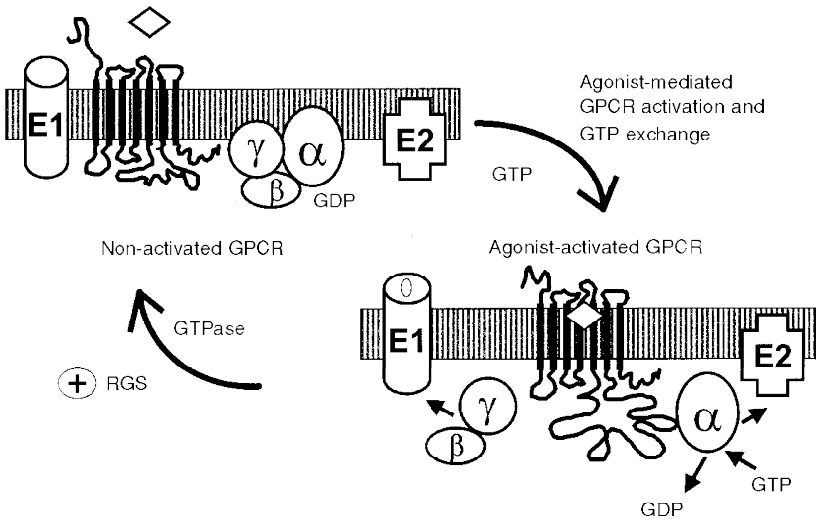

Figure 1. Schematic model of G-protein signalling cycle

In the non-activated state, the GPCR is in the inactive conformation, and GDP is bound to the heterotrimeric G-protein. Upon agonist binding, the GPCR undergoes a conformational change to the activated state and is able to bind to the heterotrimeric G-proteins and act as a guanine nucleotide exchange factor. This causes the release of GDP and allows GTP to bind to the G-protein α-subunit. Following the nucleotide exchange the G-protein can dissociate into the GTP bound α-subunits and the $\beta\gamma$-dimer. Both the α-subunit and the $\beta\gamma$-dimer can interact with effector molecules such as ion channels (E1) or membrane bound enzymes (E2) and modulate their activity. The deactivation of signalling is initiated by the hydrolysis of GTP by the α-subunit. This reaction can be accelerated by proteins termed regulators of G-protein signalling (RGS) which have been shown to directly bind to the α-subunit of G-proteins. In the GDP bound state the α-subunit reassembles with the $\beta\gamma$-dimer to form the inactive heterotrimer.

for agonist-activated GPCRs is critical for their role in initiating homologous desensitization. Desensitization is thought to be achieved when 'uncoupling' proteins such as the arrestins (Krupnick & Benovic, 1998) bind to the GRK-phosphorylated receptors and cause receptor–G-protein uncoupling by preventing further interactions of the phosphorylated GPCRs with G-proteins (Fig. 2). Interestingly, phosphorylation by other kinases does not necessarily lead to arrestin binding; for example, arrestins do not bind to β_2AR phosphorylated by protein kinase A (Lohse *et al.* 1992). Thus a second level of specificity is built into the GRK–arrestin-dependent mechanism of homologous desensitization. Four arrestins have been identified; visual and cone arrestins are localized to the visual system, whereas arrestin 2 and arrestin 3 (also referred to as β-arrestin 1 and β-arrestin 2) are ubiquitously distributed and thought to play a role in the uncoupling of many GPCRs (Krupnick & Benovic, 1998). Whether or not other uncoupling proteins exist is a topic that needs to be explored.

A second event associated with desensitization of GPCRs is internalization of GPCRs from the plasma membrane (Figs 2 and 3). Recently it has become evident that multiple pathways of internalization of GPCRs exist (Fig. 3). The most well understood is GRK and arrestin dependent. The non-visual arrestins (arrestin 2 and 3) bind to clathrin and act as adaptors to facilitate the clathrin-mediated endocytosis of select GPCRs, such as β_2ARs (Ferguson *et al.* 1996; Goodman *et al.* 1996; Zhang *et al.* 1996*a*). The arrestin- and clathrin-dependent internalization is a dynamin-dependent event (Zhang *et al.* 1996*a*) (Fig. 3). Dynamin is a GTPase that forms the necks of clathrin-coated pits and is essential for pinching off the vesicles from the plasma membrane (Urrutia *et al.* 1997). This pathway is not observed with all GPCRs, since other GPCRs are internalized via arrestin- and/or clathrin-independent pathways via proteins that can be dynamin dependent or independent (Fig. 3). Notably, these newly appreciated arrestin- and dynamin-independent pathways represent novel pathways of internalization whose components have yet to be identified. To illustrate the variations in the mechanisms of internalization, consider the multiple pathways of internalization that have been detected in studies of muscarinic cholinergic receptors (mAChRs) (Pals-Rylaarsdam & Hosey, 1997; Lee *et al.* 1998; Vogler *et al.* 1998). It is not yet clear if these pathways

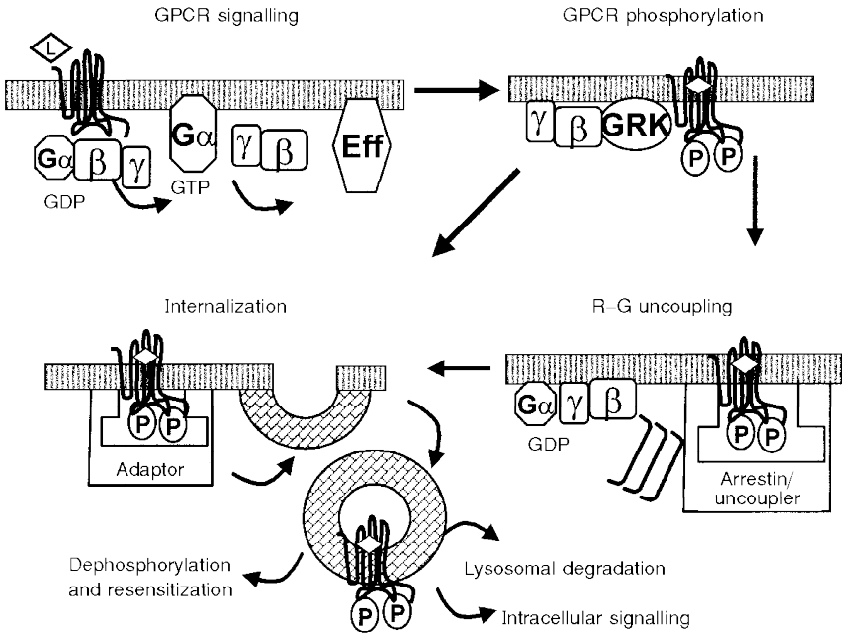

Figure 2. Scheme of GRK-dependent modulation of GPCR-mediated signalling

After activation of GPCRs by ligands (L), the receptors activate heterotrimeric G-proteins as described in Fig. 1. In the case of GRK2/3, the $\beta\gamma$-subunits of G-proteins may recruit GRKs to the membrane to allow for GPCR phosphorylation (P) of activated GPCRs. Phosphorylation by GRKs allows the GPCRs to interact with uncoupling proteins such as arrestins which cause uncoupling of the GPCRs from G-proteins. In addition to the uncoupling reaction, many GPCRs undergo internalization. This can be achieved by targeting of the phosphorylated receptor by an adaptor protein to endocytotic pathways. Internalization of the β_2AR proceeds subsequent to the R–G-uncoupling step; the adaptor is a non-visual arrestin that targets the β_2AR to clathrin-coated pits. For some GPCRs, internalization may proceed in a manner that is independent of the uncoupling reaction and may involve yet to be defined adaptors and endocytic machinery. After endocytosis, the GPCRs may undergo lysosomal degradation or recycle to the surface membrane. Eff, effector molecule.

are modulated by GRK-mediated phosphorylation of the mAChR subtypes, but it is clear that there are at least two distinct pathways of internalization of mAChR subtypes that differ from the arrestin-, dynamin- and clathrin-dependent pathway used by the β_2AR. One pathway, used by the M_2 mAChR in HEK cells, is arrestin and dynamin independent (Fig. 3) (Pals-Rylaarsdam *et al.* 1997). This pathway is greatly facilitated by agonist-dependent phosphorylation of the receptors but is not blocked by dominant negative (DN)-GRK2 (Pals-Rylaarsdam *et al.* 1995, 1997; Pals-Rylaarsdam & Hosey, 1997). Another pathway is arrestin independent but dynamin dependent and is used by the M_1, M_3 and M_4 mAChRs in HEK cells (Lee *et al.* 1998; Vogler *et al.* 1998). In considering this information, one might ask: if a receptor internalizes in an arrestin-independent manner, might the receptor–G-protein uncoupling step also be arrestin independent? Alternatively, if a receptor interacts with arrestin to cause receptor–G-protein uncoupling, why doesn't arrestin target the receptor to clathrin-coated pits for internalization? Answers to such questions will undoubtedly be forthcoming in the future and provide insights into the complex pathways mediating desensitization and internalization of GPCRs.

The roles of endocytosis in the desensitization of GPCRs also are not completely understood, and different consequences of internalization have been noted depending on the GPCR being studied and the cellular background. In certain cases, such as with the M_2 mAChRs studied in HEK cells, internalization and receptor–G-protein uncoupling have been dissociated and in these cells internalization does not cause desensitization (receptor–G-protein uncoupling) (Pals-Rylaarsdam *et al.* 1995, 1997; Pals-Rylaarsdam & Hosey, 1997). However, in Chinese hamster ovary cells, other data with M_2 mAChRs suggest that internalization is necessary for receptor–G-protein uncoupling (Tsuga *et al.* 1998*a*). These contrasting results may reflect differences in

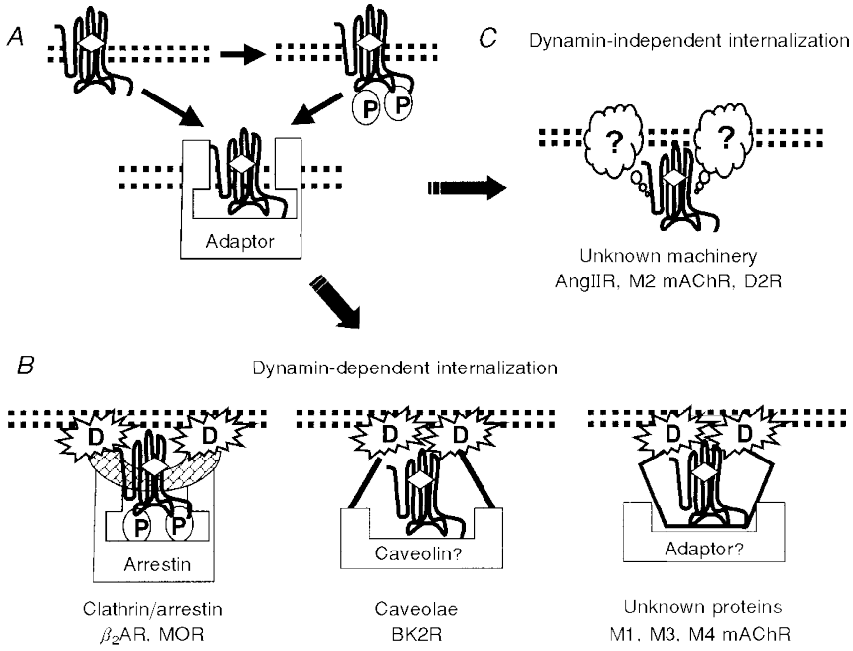

Figure 3. Multiple pathways of endocytosis for GPCRs

A, after agonist binding and activation of GPCRs, many GPCRs may undergo phosphorylation (P) by GRKs or other protein kinases. Phosphorylation can serve as a signal for the subsequent binding of certain adaptor proteins, but other mechanisms might also be operative, and might involve phosphorylation-independent binding to adaptors or direct association of the GPCRs with endocytic machinery. Subsequently, the GPCRs can be directed to different endocytic pathways. *B*, certain pathways are dynamin (D) dependent including internalization pathways that use arrestin as the adaptor and clathrin-coated pits for endocytosis as has been demonstrated for β_2-adrenergic (β_2AR) and μ-opioid receptors (MOR) (Zhang *et al.* 1997; Whistler & von Zastrow, 1998). Localization and/or internalization of GPCRs via caveolae may also occur and require dynamin for fission (Oh *et al.* 1998; Henley *et al.* 1998). For example, it has been inferred that the bradykinin 2 receptor (BK2R) is sequestered by caveolae (de Weerd & Leeb-Lundberg, 1997). It is not known if caveolin might serve as an adaptor in this process. In addition, some GPCRs, such as the M_1, M_3 and M_4 muscarinic receptors (M1, M3, M4 mAChR), may be endocytosed via a dynamin-dependent process that uses as yet unknown adaptors and/or unknown endocytic machinery (Lee *et al.* 1998). *C*, internalization of GPCRs via dynamin-independent pathways has been demonstrated for angiotensin II-1A (AngIIR), M_2-muscarinic (M2 mAChR) and D_2 dopamine (D2R) receptors (Zhang *et al.* 1996*a*; Pals-Rylaarsdam *et al.* 1997; Vickery & von Zastrow, 1999). The cellular machinery for this process is not yet known and needs to be identified in the future.

cellular machinery and different molecular pathways of desensitization. For the M_4 mAChR, internalization has been suggested to prolong desensitization (Bogatkewitsch *et al.* 1996). In marked contrast, internalization of the β_2AR appears to allow for dephosphorylation, resensitization and recycling to the plasma membrane (Pitcher *et al.* 1995; Krueger *et al.* 1997). However, not all of the internalized β_2ARs may undergo the recycling process, but rather some may undergo degradation in lysosomes, i.e. downregulation (Gagnon *et al.* 1998). The events that allow for the decision to recycle *versus* destroy are poorly understood. Recently, yet another role for internalization of GPCRs has been proposed. It has been suggested that GRK- and arrestin-dependent internalization is necessary for the β_2AR to activate the mitogen activated protein (MAP) kinase pathway via a pertussis toxin sensitive reaction (Daaka *et al.* 1998). Clearly, more studies are necessary to unravel the multiple complex mechanisms and roles of receptor internalization.

GRK structure and regulation

GRKs are 57–80 kDa proteins that are members of the large family of serine/threonine kinases. Six mammalian GRKs have been cloned so far (Palczewski, 1997; Krupnick & Benovic, 1998; Pitcher *et al.* 1998*a*). GRK1 (rhodopsin kinase) and GRK4 have been shown to be expressed in a tissue specific manner (retina and testis, respectively), and thus are expected to regulate a limited number of substrates (Palczewski, 1997). In contrast, the other GRKs are more widely distributed and evidence exists to suggest that these GRKs are likely to be involved in desensitization of multiple types of GPCRs (Palczewski, 1997).

While GRKs were initially thought to be constitutively active soluble enzymes, it is now recognized that each of the GRKs is regulated via one or more different mechanisms (for a detailed review see Palczewski, 1997), and a significant amount of the GRKs are localized to membranes (Aragay *et al.* 1998*b*; Murga *et al.* 1998). A common theme that has emerged suggests that lipid modification or interactions may be important for the membrane localization and activity of GRKs. This is achieved through several distinct mechanisms, including C-terminal palmitoylation for GRK4 and GRK6 (Loudon & Benovic, 1997), isoprenylation for GRK1 (Inglese *et al.* 1992), electrostatic binding for GRK5 (Kunapuli *et al.* 1994*a*), and by binding to membrane phospholipids (DebBurman *et al.* 1995*b*) and/or G$\beta\gamma$-subunits for GRK2 and GRK3 (Koch *et al.* 1993).

Many studies have shown that GRK1, GRK2 and GRK3 'translocate' upon activation of GPCRs (Inglese *et al.* 1994; Krupnick & Benovic, 1998). However, it is not clear whether pools of GRKs are constantly membrane associated and undergo two-dimensional rather than three-dimensional translocation upon activation of GPCRs (Palczewski, 1997). Multiple factors are likely to affect the membrane association of GRKs, such as interactions between the kinases and receptors, as the GPCRs have been shown to modulate significantly the activities of certain GRKs (Palczewski, 1997). Prenylation has been demonstrated to be required for translocation of GRK1 (Inglese *et al.* 1992), whereas translocation of GRK2 and GRK3 may involve interaction with G$\beta\gamma$ (Daaka *et al.* 1997; Krupnick & Benovic, 1998). Since GRK2 and GRK3 require the presence of negatively charged phospholipids like PIP_2 or PIP in order to be active (Onorato *et al.* 1995; DebBurman *et al.* 1996; Pitcher *et al.* 1996), it may be that G$\beta\gamma$ provides a signal for docking GRK2 and GRK3 to the plasma membrane where the kinases can bind to activating phospholipids.

The activities of GRKs appear to be inhibited by intracellular Ca^{2+} by direct interaction of GRKs with calcium-binding proteins. GRK1 can be inhibited by the calcium-binding protein recoverin (Gray-Keller *et al.* 1993; Chen *et al.* 1995), while GRK2, 5 and 6 appear to be negatively regulated by Ca^{2+}–calmodulin (Pronin *et al.* 1997). *In vitro*, the Ca^{2+}–calmodulin sensitivity of GRK5 and GRK6 is much higher than that of GRK2, suggesting that regulation by Ca^{2+}–calmodulin may be of greater physiological relevance for these kinases (Levay *et al.* 1998). In a search for additional proteins that may regulate GRKs, actin was found to bind to GRK5 (Freeman *et al.* 1998) and to inhibit the kinase activity of GRK5 in a concentration-dependent manner. Ca^{2+}–calmodulin and actin compete for inhibiting GRK5 and therefore may bind to an overlapping region on GRK5 (Freeman *et al.* 1998). Despite the biochemical evidence that Ca^{2+}–calmodulin can interact with GRKs and inhibit the phosphorylation of GPCRs, there is no direct evidence for *in vivo* regulation of GRKs by calcium. In fact, the physiological role of Ca^{2+}-dependent regulation of GRK1 has been questioned in a recent study, where no decrease in light-dependent phosphorylation of rhodopsin was found in retinas treated with α-toxin, which forms Ca^{2+}-permeable pores in membranes (Otto-Bruc *et al.* 1998). GRK2 and GRK5 are substrates for PKC (Chuang *et al.* 1995; Pronin & Benovic, 1997). However, PKC-mediated phosphorylation of GRK2 results in an activation of this kinase (Pronin & Benovic, 1997), whereas GRK5 is negatively regulated by PKC (Chuang *et al.* 1995).

Specificity of GRKs

While six members of the GRK family have been identified so far, hundreds to thousands of GPCRs are predicted to exist. Thus, one interesting question concerns the specificity of the regulation of GPCRs by GRKs. Since GRK1 and GRK4 are expressed only in retina (Zhao *et al.* 1998) and testis/kidney (Virlon *et al.* 1998), respectively, the specialized localization of these GRKs is likely to contribute to their specificities. However, the other members of the GRK family are more widely distributed, and other factors must contribute to specificity of kinase–substrate interactions. A few GPCRs appear to be selectively phosphorylated by certain GRKs. For example, endothelin receptors (ET_A and ET_B) were shown to be preferentially phosphorylated by GRK2 when expressed in HEK293 cells (Freedman *et al.* 1997), while thrombin receptors (Ishii *et al.* 1994; Iaccarino *et al.* 1998*a*), as well as odorant receptors (Peppel *et al.* 1997),

have been shown to be specifically phosphorylated and desensitized by GRK3. On the other hand, there are several receptors that appear to be phosphorylated equally well by GRK2, GRK3 and GRK5 *in vitro*, including the angiotensin II type 1a receptors (Ishizaka *et al.* 1997; Oppermann *et al.* 1996), β_2ARs (Premont *et al.* 1995; Menard *et al.* 1996) and muscarinic M_2 and M_3 receptors (Richardson *et al.* 1993; Debburman *et al.* 1995*a*). For these and other receptors that are substrates for multiple GRKs, the specificity of GRK–GPCR interaction may be defined in part by the cellular and subcellular distribution of GRKs. Some insights into this issue have been obtained in studies of transgenic mice. For example, in transgenic mice targeted to overexpress GRK2 in cardiac myocytes, a reduced responsiveness to βAR agonists and angiotensin II was observed, while mice overexpressing the C-terminal portion of GRK2 (that inhibits GRK2 by virtue of its ability to bind G$\beta\gamma$) exhibited increased sensitivity to βAR agonists (Koch *et al.* 1995; Rockman *et al.* 1996). Interestingly, mice overexpressing GRK3 showed no difference in β-adrenergic or angiotensin II signalling, while they did exhibit a decrease in thrombin receptor signalling (Iaccarino *et al.* 1998*a*). As the studies of the GRK-transgenic mice progress, it will be of interest to determine what other known substrates of the GRKs are affected by the targeted overexpression of the GRKs. In addition, it would also be of interest to determine if the effects of overexpressing the C-terminus of GRK2 are due specifically to an inhibition of GRK2 *versus* a generalized sequestration of G$\beta\gamma$-subunits and a consequent inhibition of G$\beta\gamma$-dependent signalling pathways. In other studies, long-term stimulation with β-adrenergic agonists or antagonists in mice caused upregulation or downregulation, respectively, of GRK2, but not of any other GRK, in cardiac myocytes (Iaccarino *et al.* 1998*b*), indicating that the degree of GRK specificity can be high *in vivo*, even if there is no clear specificity *in vitro*. This suggests that other, as yet undefined, factors contribute to the specificity of GRK–GPCR interactions in intact cells.

How important are GRKs for desensitization of GPCRs other than the β_2AR?

GRKs have been implicated in the initiation of agonist-induced desensitization of a variety of GPCRs (Table 1). In order to characterize the causes of desensitization, it is important to measure the desensitization of G-protein-activated signalling pathways in intact cells. However, under these conditions it is difficult to distinguish between desensitization that occurs at the level of the receptor *versus* G-proteins or effectors. To overcome this problem and to gain direct insights into the roles of GRKs in receptor desensitization, alternative approaches have been adopted to assess receptor desensitization, including the use of purified and reconstituted systems (Benovic *et al.* 1987*b*; Kwatra *et al.* 1989*a*; Lohse *et al.* 1992; Richardson *et al.* 1993), where GRK-mediated phosphorylation and its functional consequences can be monitored directly, as well as the use of isolated cell membranes to assess the ability of GRKs to modulate receptor stimulated G-protein activity (Oppermann *et al.* 1996; Whistler & von Zastrow, 1998). In addition, a large number of studies have used overexpression of GRKs and/or arrestins to assess the participation of these proteins in desensitization and internalization of GPCRs. Notably, the measurement of agonist-induced internalization of GPCRs has frequently been used as an 'indicator' of receptor desensitization, and, most recently, many investigators have asked if receptor internalization is promoted by GRKs (Tsuga *et al.* 1994, 1998*c*; Zhang *et al.* 1998). However, caution needs to be exercised in those cases where receptor internalization is used to reflect receptor desensitization for several reasons. First, the actual desensitization of GPCRs, i.e. the uncoupling of receptors from G-proteins, often precedes sequestration and internalization of these receptors (Roth *et al.* 1991). In addition, there are cases where receptor–G-protein uncoupling appears to be mediated by distinct mechanisms from those that promote receptor internalization (Pals-Rylaarsdam *et al.* 1995, 1997; Pals-Rylaarsdam & Hosey, 1997). Indeed, internalization can occur in the absence of receptor–G-protein uncoupling (Pals-Rylaarsdam & Hosey, 1997). It is important to keep in mind that the mechanisms and roles of internalization have not been completely defined and cannot be assumed to reflect directly the mechanisms promoting receptor– G-protein uncoupling.

GRKs can phosphorylate a variety of GPCRs *in vitro* and/or in native systems or in heterologous expression systems (Table 1). Overexpression of GRKs was shown to enhance internalization and/or desensitization of many GPCRs, but this does not necessarily mean that these receptors normally utilize the GRK pathway defined for the β_2AR for their desensitization and/or internalization. It is important to distinguish between desensitization events that occur with endogenous cellular machinery and those that may be 'forced' to occur by overexpression of GRKs and/or arrestins, as multiple pathways of desensitization and internalization are likely to exist.

In order to gain insights into the type of pathway that is utilized for desensitization some useful tools and approaches are available. The availability of purified GRKs allows one to ask if a given receptor is a substrate for GRKs *in vitro*. Typically such assays are performed using purified GRKs and purified and reconstituted receptors (Benovic *et al.* 1987*a*, *b*; Richardson & Hosey, 1992; Richardson *et al.* 1993; Haga *et al.* 1996), although other approaches using membranes have also been introduced (Pei *et al.* 1994; Debburman *et al.* 1995*a*). If the receptor is found to be stoichiometrically phosphorylated *in vitro* with 'reasonable' concentrations of receptor and GRK, then this receptor may be a potential target of GRK in intact cells. Unfortunately there are no specific pharmacological inhibitors of GRKs available that can be used in intact cells to corroborate results that have obtained from *in vitro* phosphorylation studies.

Another approach to determine if GRKs participate in receptor regulation is to ask if a loss of GRK activity causes a loss of receptor phosphorylation, desensitization and/or internalization. For example, one can ask if overexpression of a dominant negative (DN) GRK (Kong *et al.* 1994) inhibits the phosphorylation, desensitization and/or internalization that is observed in response to agonist. If the answer is yes, this is considered to be a good indication for a GRK-dependent desensitization process. However, there are several caveats to be considered. Overexpression of DN GRK2 or 3 can cause a sequestering of G$\beta\gamma$ in these cells and therefore will inhibit signalling mediated by the G-protein $\beta\gamma$-subunits (Inglese *et al.* 1994). In fact, overexpression of the C-terminus ($\beta\gamma$-binding domain) of GRK2 has been demonstrated to disrupt G-protein-mediated signalling such as MAP kinase activation via stimulation of M_1 and M_2 mAChRs (Crespo *et al.* 1994) or M_2 mAChR-mediated activation of G$\beta\gamma$-activated GIRK channels (Bünemann & Hosey, 1998). Therefore it is very important to also use as a control overexpressed wild-type (WT) GRK2 or 3 and to compare and contrast the effects of the WT and DN GRKs to differentiate between effects due to the kinase activity of GRK2 or 3 and their G$\beta\gamma$-sequestering activity. Overexpression of WT GRKs might increase phosphorylation, desensitization and/or internalization of a GPCR. In this scenario, the results would suggest that the GPCR under study is *capable* of undergoing regulation by GRKs, but it would not necessarily suggest that this occurs physiologically. Such results could suggest that a particular cell has a low complement of GRKs, or that the GPCR is a relatively poor substrate for GRKs. Another approach to explore the function of GRKs in the regulation of GPCRs in intact cells is to use strategies that knock out or at least reduce the amount of GRKs. The development of GRK knock-out mice (discussed below) and antisense approaches are of great potential. These approaches are less prone to the problems that overexpression of signalling molecules can cause, i.e. pushing a signalling pathway in a new direction.

In order to test whether arrestins are important for desensitization and internalization of GPCRs, several useful arrestin constructs have been developed that may help to clarify this issue. Increased internalization due to the overexpression of WT arrestins would indicate that a given GPCR can be internalized in an arrestin-dependent manner, but would not indicate that the endogenous internalization pathway is arrestin dependent. However, overexpression of the clathrin-binding domain of arrestin 2 or 3 blocks internalization of β_2AR and may be a useful tool to determine if other GPCRs are internalized via arrestin–clathrin interaction (Fig. 2) (Krupnick *et al.* 1997; Zhang *et al.* 1997). On the other hand, there are instances where overexpression of neither WT nor DN arrestins affected internalization (Lee *et al.* 1998), suggesting that the internalization of certain GPCRs is insensitive to arrestins. Similar problems as discussed for overexpression of GRK constructs may occur with overexpression of arrestins, and therefore knock-out or antisense approaches may help to determine which role arrestins play *in vivo*. Recently an antisense approach has yielded selected clones of HEK293 cells with reduced expression of arrestin 2 and arrestin 3 (J. L. Benovic, personal communication). In these cells, β_2ARs exhibited less internalization than in control cells.

Another important tool to dissect the pathway of receptor internalization is a dominant negative mutant of dynamin, dynamin K44A (Damke *et al.* 1994), which is deficient in GTPase activity and unable to catalyse the fission of clathrin-coated pits from the membrane. This protein has been shown to inhibit endocytosis via clathrin-coated pits in many different systems (van der Bliek *et al.* 1993; Baba *et al.* 1995; Zhang *et al.* 1996*a*; Altschuler *et al.* 1998). In addition, the DN dynamin mutant also has been recently reported to inhibit the pinching off of caveolae (Henley *et al.* 1998; Oh *et al.* 1998), thus demonstrating that caveolae also use a dynamin-dependent process (see Fig. 3). The B2 bradykinin receptor has been reported to be sequestered in caveolae in response to agonist treatment (de Weerd & Leeb-Lundberg, 1997). The M_1, M_3 and M_4 mAChRs are internalized in HEK cells in a dynamin-dependent manner (Lee *et al.* 1998; Vogler *et al.* 1998), but the internalization of these receptors is not inhibited by dominant negative arrestin mutants (Lee *et al.* 1998). Therefore these receptors do not appear to be internalized via the arrestin–clathrin pathway used by β_2ARs, but they could conceivably be internalized via caveolae or, alternatively, may use other adaptor proteins that may target these receptors to clathrin-coated pits or as yet unidentified endocytic vesicles (Fig. 3).

Importance of GRKs in desensitization *in vivo*

Studies in heterologous expression systems have provided insights into the mechanisms involved in desensitization and internalization. However, in many cases it is difficult to test whether the desensitization pathway that is utilized in heterologous expression systems is comparable to that utilized in native tissues. Technical limitations, such as difficulties in transfecting native cells with plasmids carrying the cDNAs for various proteins of interest, hamper our current understanding of the mechanisms of desensitization in native systems and *in vivo*. However, recent insights into the roles of GRKs *in vivo* have been provided from studies of transgenic mice. Homozygous GRK2 knock-out mice were embryonic lethal at embryonic day 15. The embryos had severe defects in cardiac development and most probably died of cardiac failure (Jaber *et al.* 1996), suggesting an unknown but important role for GRKs in cardiac development. In contrast the heterozygous GRK2(+/−) mice were normal and these mice, as well as GRK2(+/−) mice overexpressing the C-terminus of GRK2 ('GRK2 inhibitor'), exhibited increased contractile function compared with control mice (Rockman *et al.* 1998*b*). Furthermore, as discussed above, mice overexpressing GRK2 showed a reduced responsiveness to β_2AR stimulation, while animals overexpressing the C-terminal portion of GRK2

Table 1. Evidence for GRK-dependent regulation of G-protein coupled receptors

		GRK			Internalization	
G-protein coupled receptors	Agonist-dependent phosphorylation	*In vitro* phosphorylation by	*In vivo* effects of	DN GRK2 sensitive	Arrestin dependent	Dynamin dependent
A_1-Purinergic	*In vitro* (Ramkumar *et al.* 1993); *in vivo* (Ciruela *et al.* 1997)	GRK2, 3 (Ramkumar *et al.* 1993)				
A_2A/A_2B Purinergic	*In vivo* (Palmer & Stiles, 1997)		GRK2 DES (Palmer & Stiles, 1997; Mundell *et al.* 1998)	DES inhibited (Mundell *et al.* 1997)		
A_3-Purinergic	*In vitro* and *in vivo* (Palmer *et al.* 1995)	GRK2 (Palmer *et al.* 1995)				
α_2-Adrenergic	*In vivo* (α_{2A}-AR) (Liggett *et al.* 1992)	GRK2 (Benovic *et al.* 1987*b*)	P, DES (Liggett *et al.* 1992)			
α_{1B}-Adrenergic	*In vivo* (Lattion *et al.* 1994, Diviani *et al.* 1996, 1997)		GRK2, 3, 6 P (Diviani *et al.* 1996, 1997) GRK2, 3, DES (Diviani *et al.* 1996)	P inhibited (Diviani *et al.* 1996)		
Angiotensin II 1a	*In vivo* (Oppermann *et al.* 1996)		GRK2 P (Oppermann *et al.* 1996)	P, DES inhibited (Oppermann *et al.* 1996)	No (Zhang *et al.* 1996*a*)	No (Zhang *et al.* 1996*a*)
β_1-Adrenergic	*In vivo* and *in vitro* (Freedman *et al.* 1995)	GRK2, 3, 5 (Freedman *et al.* 1995)	GRK1, 2, 3, 5 P, DES (Freedman *et al.* 1995)	P inhibited (Freedman *et al.* 1995)	(DES) arrestin 2, 3 dependent (Freedman *et al.* 1995)	
β_2-Adrenergic	*In vivo* (Sibley *et al.* 1986; Strasser *et al.* 1986; Fredericks *et al.* 1996; January *et al.* 1997); *in vitro* (Benovic *et al.* 1986)	GRK2, 3, 4, 5, 6 (Benovic *et al.* 1991; Pitcher *et al.* 1992; Benovic & Gomez, 1993; Kunapuli *et al.* 1994*b*; Fredericks *et al.* 1996; Premont *et al.* 1996)	GRK2 P, DES (Hausdorff *et al.* 1990) GRK2 DES (Koch *et al.* 1995) GRK2 I (Menard *et al.* 1996)	P inhibited (Kong *et al.* 1994) DES (Kong *et al.* 1994)	Yes (Zhang *et al.* 1997)	Yes (Zhang *et al.* 1997)

CCR-5 chemokine	*In vitro* (Aramori *et al.* 1997)		GRK2, 3 P, Des (Aramori *et al.* 1997)		Yes arrestin 2, 3 (Aramori *et al.* 1997)	Yes (Aramori *et al.* 1997)
D_1A-dopamine	*In vivo* (Ng *et al.* 1994; Tiberi *et al.* 1996)		GRK2, 3, 5 P, DES (Tiberi *et al.* 1996)			
$ET_{A,B}$-endothelin	$ET_A + ET_B$ (yes) *in vivo* (Freedman *et al.* 1997) ET_A (yes), ET_B (no) *in vivo* (Cramer *et al.* 1997)		GRK2, 5, 6 P (Freedman *et al.* 1997) GRK2 DES (Freedman *et al.* 1997)	P, DES inhibited (Freedman *et al.* 1997)		
N-Formyl peptide	*In vivo* (Hsu *et al.* 1997; Prossnitz, 1997); *in vitro* (Prossnitz *et al.* 1995)	GRK2, 3 (Prossnitz *et al.* 1995)				
Follitropin	*In vivo* (Quintana *et al.* 1994; Nakamura *et al.* 1998)				Yes (Nakamura *et al.* 1998)	
GnRH-R			GRK2, 3, 6 DES (Neill *et al.* 1998)		(Des) increased by arrestin 2, 3 (Neill *et al.* 1998)	
Lutropin/ choriogonadotropin	*In vivo* (Hipkin *et al.* 1993, 1995; Wang *et al.* 1997; Lazari *et al.* 1998)		GRK4 DES (Premont *et al.* 1996)		Yes, arrestin 2 (Lazari *et al.* 1998)	Yes (Lazari *et al.* 1998)
M_1 muscarinic	*In vitro* (Haga *et al.* 1996)	GRK2, 3 (Haga *et al.* 1996)	GRK2 I not affected (Tsuga *et al.* 1998*c*)	I not inhibited (Tsuga *et al.* 1998*c*)	No (Lee *et al.* 1998)	Yes (Lee *et al.* 1998; Vogler *et al.* 1998)
M_2 muscarinic	*In vivo* (Kwatra & Hosey, 1986; Kwatra *et al.* 1987, 1989*b*; Richardson & Hosey, 1992); *in vitro* (Kwatra *et al.* 1989*a*)	GRK2, 3, 5, 6 (Kwatra *et al.* 1989*a*; Kunapuli *et al.* 1994*b*; Loudon & Benovic, 1994; Richardson *et al.* 1993)	GRK2, 3 I (Schlador & Nathanson, 1997; Tsuga *et al.* 1998*a*, *b*, *c*) GRK2, 3 DES (Schlador & Nathanson, 1997)	P, DES inhibited (Pals-Rylaarsdam *et al.* 1995) I inhibited (Tsuga *et al.* 1994) I not inhibited (Pals-Rylaarsdam *et al.* 1995)	Yes, arrestin 2, 3 (Schlador & Nathanson, 1997); dependent + independent (Pals-Rylaarsdam *et al.* 1997)	No (Pals-Rylaarsdam *et al.* 1997; Vogler *et al.* 1998)
M_3 muscarinic	*In vitro* (Debburman *et al.* 1995*a*); *in vivo* (Tobin *et al.* 1995)	GRK2, 3 (Debburman *et al.* 1995*a*)	GRK2 I (Tsuga *et al.* 1998*c*)	I not inhibited (Tsuga *et al.* 1998*c*)	No (Lee *et al.* 1998)	Yes (Lee *et al.* 1998; Vogler *et al.* 1998)

Table 1 continued

G-protein coupled receptors	Agonist-dependent phosphorylation	GRK: *In vitro* phosphorylation by	GRK: *In vivo* effects of	GRK: DN GRK2 sensitive	Internalization: Arrestin dependent	Internalization: Dynamin dependent
M_4 muscarinic			GRK2 I (Tsuga *et al.* 1998*c*)	I inhibited (Tsuga *et al.* 1998*c*)	No (Lee *et al.* 1998)	Yes (Lee *et al.* 1998; Vogler *et al.* 1998)
CCR-2B chemokine	*In vivo* (Aragay *et al.* 1998*a*)		GRK3 P, DES, I (Franci *et al.* 1996) GRK2, DES (Aragay *et al.* 1998*a*)	DES inhibited (Aragay *et al.* 1998*a*)		
Neurokinin-1 (substance P)	*In vitro* (Kwatra *et al.* 1993; Nishimura *et al.* 1998)	GRK2, 3 (Kwatra *et al.* 1993; Nishimura *et al.* 1998)			Colocalization (McConalogue *et al.* 1998)	
Odorant	*In vivo* (Schleicher *et al.* 1993)		Knock-out of GRK3 lacks DES (Peppel *et al.* 1997)		(DES) increased by arrestin 3 (Dawson *et al.* 1993)	
μ-Opioid	*In vivo* (Arden *et al.* 1995; Zhang *et al.* 1996*b*; Yu *et al.* 1997)		GRK2 P, DES (Zhang *et al.* 1998) GRK2, 5 DES (Kovoor *et al.* 1998)		(DES) increased by arrestin 3 (Kovoor *et al.* 1998; Whistler & von Zastrow, 1998), not by arrestin 2 (Cheng *et al.* 1998)	Yes (Whistler & von Zastrow, 1998)
δ-Opioid	*In vivo* (Pei *et al.* 1995)		GRK2, 5 P (Pei *et al.* 1995; Hasbi *et al.* 1998) GRK2 DES (Pei *et al.* 1995)	P, DES inhibited (Pei *et al.* 1995)	(DES) increased by arrestin 2 (Cheng *et al.* 1998)	Yes (Chu *et al.* 1997; Murray *et al.* 1998)
κ-Opioid	*In vivo* (Appleyard *et al.* 1997)				(DES) increased by arrestin 2 (Cheng *et al.* 1998)	No (Chu *et al.* 1997)

Platelet activating factor	*In vivo* (Takano *et al.* 1994; Ishii *et al.* 1998)	GRK2 P (Ishii *et al.* 1998)	
Thrombin	*In vivo* (Brass, 1992; Ishii *et al.* 1994; Vouret-Craviari *et al.* 1995)	GRK3 DES (Ishii *et al.* 1994) GRK3 translocation (Daaka *et al.* 1997), transgenic mice overexpressing GRK3 exhibit attenuated thrombin signalling (Iaccarino *et al.* 1998*a*)	
Thyrotropin (TSH)		GRK2, 5 DES (Iacovelli *et al.* 1996; Nagayama *et al.* 1996)	(DES) increased by arrestin 2 (Iacovelli *et al.* 1996)
V_1 vasopressin	*In vivo* (Innamorati *et al.* 1998*a*)	Undefined GRKs P (Innamorati *et al.* 1998*a*)	
V_2 vasopressin	*In vivo* (Innamorati *et al.* 1997)	Undefined GRKs P (Innamorati *et al.* 1997, 1998*b*)	

The first column provides a partial list of GPCRs that have been analysed as targets of GRK-dependent regulation. Studies that demonstrated agonist-dependent phosphorylation of G-protein coupled receptors either in cell-free assays (*in vitro*) or in intact cells (*in vivo*) are listed in column 2. GRKs that have been demonstrated to phosphorylate receptors in cell-free assays are listed in column 3, whereas effects of heterologously expressed GRKs on agonist-induced desensitization (DES), phosphorylation (P) and internalization (I) of individual GPCRs in intact cells are shown in column 4. The last two columns cite studies that have analysed the effects of coexpression of arrestins or a dominant negative mutant of dynamin on agonist-induced internalization of given GPCRs. If coexpression of arrestins increased, or if dominant negative dynamin inhibited, agonist-induced internalization of receptors it is stated with 'yes', if not it is stated with 'no'. Studies that observed an increase of desensitization of the GPCR upon coexpression of arrestins are indicated as (DES). GnRH, gonadotropin-releasing hormone.

('βARK-inhibitor') exhibited increased βAR responsiveness (Koch *et al.* 1995). Recently, the decreased myocardial βAR responsiveness that was observed in animal models of chronic heart failure, as well as in human hearts (Bristow *et al.* 1990), has been correlated with increased GRK activity (most likely to be GRK2 and GRK5) (Ungerer *et al.* 1993, 1994). In addition, targeted overexpression of the 'GRK2 inhibitor' has been demonstrated to prevent the development of cardiomyopathy in a murine model of heart failure (Rockman *et al.* 1998*a*). These and other studies that demonstrated that changes of the activity of GRKs in myocytes resulted in alteration of the βAR responsiveness suggest that GRKs might actually play important roles in regulating cardiac contractility *in vivo* (Koch *et al.* 1995; Ungerer *et al.* 1996; Akhter *et al.* 1997; Drazner *et al.* 1997). Reducing GRK activity *in vivo*, for example by gene transfer, could be a potential new therapeutic approach for patients with chronic heart failure (Drazner *et al.* 1997; Rockman *et al.* 1998*a*).

Another successful strategy has been to document the pathophysiology caused by naturally occurring mutations in the *GRK1* gene. Mutations in the *GRK1* gene have been correlated with Oguchi disease, an autosomal recessive form of stationary night blindness in man characterized in part by delayed photoreceptor recovery (Yamamoto *et al.* 1997). Biochemical studies have confirmed that these mutations lead to gene products that virtually lack GRK1 activity (Khani *et al.* 1998). Thus the lack of GRK1 results in lack of the normal 'turn-off' of rhodopsin and the consequent disease state.

Role of GRKs in regulating M_2 mAChRs

While it is not possible to review extensively in this article the evidence for a role of GRKs in the regulation of very many GPCRs, a non-visual, non-adrenergic GPCR that has been analysed fairly extensively in terms of desensitization is the M_2 mAChR. GRKs have been implicated in the phosphorylation and desensitization of M_2 mAChR, and *in vitro* these receptors are comparable as substrates for GRK2, 3 and 5 to the β_2AR (Kwatra *et al.* 1989*a*; Richardson & Hosey, 1992; Kunapuli *et al.* 1994*b*; Tsuga *et al.* 1994; Pals-Rylaarsdam *et al.* 1995). Agonists are known to induce phosphorylation and desensitization of the M_2 mAChR in intact cardiac myocytes (Kwatra & Hosey, 1986; Kwatra *et al.* 1987, 1989*b*). While no direct evidence links GRKs to this reaction, the properties of agonist-induced phosphorylation of the M_2 mAChR in cardiac myocytes closely resemble the GRK-mediated phosphorylation and regulation of these receptors that is observed in heterologous expression systems and in reconstituted systems (Kwatra *et al.* 1989*a*; Richardson & Hosey, 1992; Richardson *et al.* 1993; Pals-Rylaarsdam *et al.* 1995). In addition, in HEK cells, DN GRK2 reduced agonist-dependent phosphorylation of the M_2 mAChR by 50% and eliminated desensitization of the G_i-mediated attenuation of adenylyl cyclase (Pals-Rylaarsdam *et al.* 1995), suggesting that an endogenous GRK regulates the M_2 mAChR in these cells. On the other hand, in the same cell system, overexpression of DN GRK2 or WT GRK2 had no effect on internalization of the M_2 mAChR, suggesting that GRKs may not play a role in the internalization of these receptors in HEK cells. In similar studies a small effect of WT GRK on internalization was observed in CHO-K1 cells, but only at low concentrations of agonist (Tsuga *et al.* 1994).

The M_2 mAChR also activates G-protein gated K^+ (K_{ACh}) channels in cardiac atrial myocytes, and this Gβγ-mediated response rapidly desensitizes (Yamada *et al.* 1998). Treatment of isolated atrial myocytes with muscarinic agonists for seconds to several minutes causes heterologous desensitization of the ability of other endogenous G_i-coupled receptors, such as the A_1 adenosine or sphingosylphosphorylcholine receptors, to activate this current (Bünemann *et al.* 1997). In addition, homologous desensitization of ACh-activated K_{ACh} channels occurs in atrial myocytes with a time course of several minutes to many hours (Shui *et al.* 1995, 1998; Bünemann *et al.* 1996). When freshly isolated atrial myocytes are cultured in the absence of ACh, an increase in sensitivity to ACh is observed, suggesting that atrial myocytes might be partially desensitized *in vivo* (Bünemann *et al.* 1997). Further support for this suggestion is that the time course of the development of the increase in agonist sensitivity during agonist-free culture was similar to that of the time course of recovery of the M_2 mAChR following long-term desensitization *in vitro* (Bünemann *et al.* 1997).

Other studies have implicated GRKs in these processes of desensitization. One study has shown a loss of desensitization of K_{ACh} channels in outside-out patches of rat atrial myocytes that can be restored by application of purified GRK2 (Shui *et al.* 1998). Further studies using membrane patches of CHO-K1 cells which heterologously expressed K_{ACh} channels showed that coexpression of GRK2 greatly increased the M_2 mAChR-induced desensitization of K_{ACh} channels. However, expression of the DN GRK2 also enhanced desensitization compared with cells not expressing GRK2 (Shui *et al.* 1998). Since very little desensitization was observed when neither WT nor DN GRK2 was expressed (Shui *et al.* 1998), the 'desensitization' might have been due to sequestration of Gβγ. In addition, in these studies one cannot distinguish between desensitization of the M_2 mAChR and desensitization of G-proteins or channels. However, support for a role for GRKs in the desensitization of M_2 mAChR-induced K_{ACh} channels came from observations that when DN GRK2 was overexpressed with phosphorylation-deficient mutants of the M_2 mAChR in CHO cells, agonist-dependent desensitization was reduced compared with that observed in cells expressing WT M_2 mAChR and either WT or DN GRK2 (Shui *et al.* 1998). When all the evidence is reviewed about desensitization of M_2 mAChR responses in

cardiac myocytes, the suggestion that GRKs and receptor phosphorylation may participate in desensitization is still at the correlative stage, and more studies will be required to elucidate the exact pathway(s) that lead to homologous and heterologous desensitization, as well as internalization of the M_2 mAChR in native systems.

Other functions of GRKs

Since GRKs are believed to be activated by agonist-activated GPCRs and downstream events, it is conceivable that the activated GRKs may play unknown roles in signal transduction pathways by phosphorylating non-GPCR substrates. In this sense the GRKs may function like other protein kinases that are activated by receptor and G-protein signalling. Recently several groups independently detected that GRK2 and GRK5 (Carman *et al.* 1998) can bind and phosphorylate tubulin (Carman *et al.* 1998; Haga *et al.* 1998; Pitcher *et al.* 1998*b*). The physiological consequence of the interaction of GRK2 with tubulin is not yet understood, but GRK2 does colocalize with microtubules in intact cells (Pitcher *et al.* 1998*b*) and the colocalization of GRK2 and tubulin is facilitated by activation of various GPCRs with agonist. Since tubulin is an important protein for cytoskeletal function, it is easy to speculate that GRKs may play a role in a GPCR-induced modulation of cytoskeletal arrangement. Furthermore, since receptor-induced activation of GRKs, at least for GRK1, 4, 5, and 6, seems to be independent of the activation of G-proteins, these GRKs may work themselves as signal transducers for GPCRs.

One interesting structural feature of GRKs is an RGS homology domain (Berman & Gilman, 1998) at the N-terminus of these proteins. Since RGS proteins can bind to Gα-subunits and accelerate GTP hydrolysis (i.e. they are GAPs), they play an important role in modulating G-protein signalling (Berman & Gilman, 1998; Zerangue & Jan, 1998). So far there is no experimental evidence that GRKs themselves exhibit GAP activity towards Gα-subunits, but the presence of RGS-like domains in GRKs leads to the speculation that GRKs may have additional functions in G-protein signalling. In this regard, a novel GRK-interacting protein (GIT1) has been discovered in a yeast two-hybrid screen and can bind to GRK2, 3, 5 and 6 (Premont *et al.* 1998). Overexpression of GIT1 increased GRK2-dependent phosphorylation of β_2ARs and caused receptor–G-protein uncoupling, but GIT1 also inhibited agonist-induced sequestration and resensitization of these receptors (Premont *et al.* 1998). Interestingly GIT1 was found to act as a GAP for the ARF family of small G-proteins, which are implicated in the regulation of vesicular trafficking including endocytosis. Therefore, GIT1 might be an inhibitor of endocytic pathways. If this hypothesis is right it would explain why GIT1 decreased agonist-induced internalization of β_2ARs despite the observed increase in phosphorylation (Premont *et al.* 1998). It is obvious that we have not learned all there is about the roles of GRKs in physiology and that the next years promise to provide new insights into the complex regulation of GPCRs and other functions by GRKs.

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Corresponding author

M. M. Hosey: Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, 303 E. Chicago Avenue S215, Chicago, IL 60611, USA.

Email: mhosey@nwu.edu