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PERSPECTIVE

Novel Features of G Protein-Coupled Receptor Kinase 4

Kim A. Neve

Department of Behavioral Neuroscience, Oregon Health & Science University; and Veterans Affairs Medical Center, Portland, Oregon

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ABSTRACT

The defining characteristic of G protein-coupled receptor homologous desensitization is that the receptor must be occupied by an agonist or in an activated conformation that mimics an agonist-induced state. In most instances, the mechanistic basis for this characteristic is the high selectivity of G protein-coupled receptor kinases for the activated receptor. In this issue, Rankin et al. (p. 759) demonstrate that under some conditions, at least, the G protein-coupled receptor kinase GRK4 does not display a preference for the agonist-occupied D_1 dopamine receptor. Coexpression of GRK4 and the D_1 receptor in a heterologous system induces phosphorylation of the receptor in the absence of agonist, causing constitutive desensitization and internalization of the receptor. Lacking the normal rapid feedback mechanisms associated with homologous desensitization, a system incorporating constitutively active GRK4 will be prone to dysregulation, perhaps explaining the generally low expression of GRK4. Indeed, considerable evidence suggests that just such dysregulation resulting from mutationally activated GRK4 contributes to the heritable component of human essential hypertension (*Physiol Genomics* **19:**223–246, 2004).

G protein-coupled receptors (GPCRs) typically respond to agonist stimulation with a time-dependent, rapidly reversible diminution, or desensitization, of the signaling response. Desensitization can be homologous (a receptor desensitized as a result of its own activation) or heterologous (one receptor desensitized as a consequence of the activation of and signaling by a different receptor). The canonical model of homologous desensitization of GPCRs (Van Koppen and Jakobs, 2004) is that the agonist-activated receptor binds and activates a GPCR-selective kinase (GRK) (Benovic et al., 1986), which phosphorylates the receptor on multiple serine/threonine residues. Activation and phosphorylation of the GPCR increases its affinity for arrestin (Benovic et al., 1987). Binding of arrestin to the intracellular loops of the GPCR both sterically hinders the interaction of receptor and G protein and recruits the GPCR into clathrin-coated pits for dynamin-dependent internalization into clathrin-coated vesicles (Goodman et al., 1996). The internalized GPCR may be dephosphorylated and rapidly recycled to the plasma membrane (Pippig et al., 1995) or retained in the cell and ultimately degraded, with the choice determined by factors such as the phosphorylation state of other residues (Mason et al., 2002) and the stability of the interaction between the GPCR and arrestin (Oakley et al., 1999; Pan et al., 2003).

This model of homologous desensitization has been validated for countless GPCRs. Still, at almost every step of this process, there are examples of exceptions to and deviations from the model. In some cases, the deviations represent expanded roles for some of the players, such as GRKs (Fig. 1); in other cases, the deviations represent alternative pathways in addition to those in the canonical pathway. In still other cases, it seems that a receptor uses an alternative mechanism instead of the canonical pathway. The GRK can bind to the receptor and influence signaling without phosphorylating the receptor (Perroy et al., 2003; Dhami et al., 2005), GRKs phosphorylate other proteins constitutively or upon activation by a GPCR (Pitcher et al., 1998b; Hall et al., 1999; Pronin et al., 2000), arrestin can bind to unphosphorylated receptor (Chen et al., 2004; Kim et al., 2004; Jala et al., 2005), GPCR-activated arrestin can mediate GPCR signaling in addition to internalization (Luttrell and Lefkowitz, 2002; Gurevich and Gurevich, 2003), desensitization and internal-

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ABBREVIATIONS: GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; βARK, β-adrenergic receptor kinase; HEK, human embryonic kidney.

ization can occur without phosphorylation of the GPCR (Malecz et al., 1998) or without arrestin binding (Pals-Rylaarsdam et al., 1997; Bennett et al., 2001; Bhatnagar et al., 2001; Van Koppen and Jakobs, 2004), GPCRs can internalize via clathrin- and dynamin-independent pathways (Pals-Rylaarsdam et al., 1997; Vickery and von Zastrow, 1999), and dephosphorylation/resensitization can occur in the plasma membrane without receptor internalization (Gardner et al., 2001).

One component of the model that has been constant, and that defines homologous desensitization, has been the strong preference of GRKs for phosphorylation of the agonist-activated receptor over the inactive receptor. The instances when a GPCR is robustly phosphorylated by a GRK without being occupied by an agonist are examples that prove the rule, because the receptor invariably has a high level of inherent or mutationally induced constitutive activity (Ren et al., 1993; Pei et al., 1994; Geras-Raaka et al., 1998; Miller et al., 2003; Marion et al., 2004); the GPCR must be in an active conformation for significant GRK-catalyzed phosphorylation to occur. There is also some evidence that a GRK bound to an activated GPCR might phosphorylate adjacent, inactive GPCRs (Palczewski, 1997), but that does not invalidate the fundamental requirement for an activated receptor to bind and activate the GRK.

In this issue of *Molecular Pharmacology*, Rankin et al. (2006) demonstrate that heterologously expressed dopamine D_1 receptor is constitutively phosphorylated by heterologously expressed GRK4. The D_1 receptor does not have an unusually high level of constitutive activity, yet when coexpressed in HEK293 cells with GRK4, it is phosphorylated to such an extent that agonist treatment causes little additional phosphorylation; in this system, GRK4 apparently does not distinguish between active and inactive D_1 receptor. GRK4-catalyzed constitutive phosphorylation is associated with reduced dopamine-stimulated cyclic AMP accumulation (desensitization) and receptor internalization. The constitutively phosphorylated residues seem to be near the C terminus of the receptor, because truncation at residue Thr404 or combined mutation of Thr428 and Ser431 prevents or substantially decreases constitutive phosphorylation, desensitization, and internalization of the receptor. Thus, the inactive D_1 receptor can be phosphorylated by GRK4, resulting in diminished responsiveness to subsequent stimulation by dopamine.

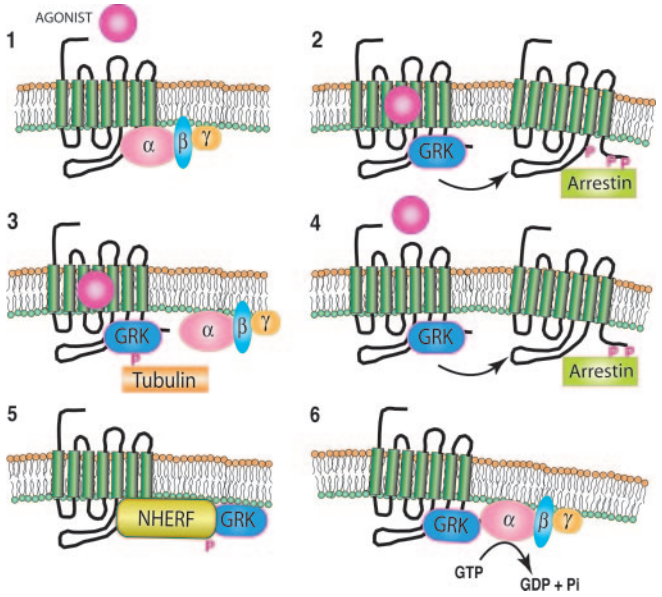

Fig. 1. Expanded role for G protein-coupled receptor kinases in desensitization and signaling. 1, a GPCR is shown precoupled to a heterotrimeric G protein before activation by agonist. 2, in the canonical model of homologous desensitization, the agonist-activated GPCR binds to and is phosphorylated by GRK, which promotes the binding of arrestin, thus interfering with coupling to the G protein. 3, GRK binds to the receptor and noncatalytically hinders GPCR coupling to the G protein. Receptor-activated GRK also phosphorylates other proteins, such as tubulin. 4, Rankin et al. (2006) demonstrate that GRK4 constitutively phosphorylates a nonagonist-occupied GPCR, leading to desensitization and internalization, presumably via binding of arrestin. 5, GRK constitutively phosphorylates other receptor-interacting proteins, such as the Na^+/H^+ exchanger regulatory factor (NHERF). 6, GRK2/3, in particular, may also decrease signaling by binding to Gα and accelerating its GTPase activity (Willets et al., 2003), stimulating the hydrolysis of GTP to GDP and inorganic phosphate.

The seven GRKs are grouped into three subfamilies: the retinal subfamily (GRK1/7), the β-adrenergic receptor kinase (βARK) subfamily (GRK2/3), and the GRK4 subfamily (GRK4/5/6). Characteristics of the GRK4 subfamily include predominant localization at the membrane as a result of palmitoylation on C-terminal cysteine residues (for GRK4/6) or interaction between a positively charged domain near the C terminus and negatively charged membrane phospholipids (GRK5), activation by phosphatidylinositol bisphosphate binding to an N-terminal domain, and enhanced sensitivity to inhibition by calcium-sensor proteins such as calmodulin (Pronin et al., 1997; Pitcher et al., 1998a; Kohout and Lefkowitz, 2003; Willets et al., 2003). GRK4 is unusual within its subfamily (but similar to GRK1) in that its relatively low sequence homology across species suggests that it is subject to lower evolutionary pressure for sequence conservation and evolving more rapidly than the other members of its subfamily and the βARK subfamily (Premont et al., 1999). It is interesting that GRK1/7 and GRK4 also differ from the other GRKs in tissue distribution. GRK2/3 and GRK5/6 are ubiquitously expressed, whereas GRK1/7 are expressed almost exclusively in the retina, and GRK4 is abundantly expressed only in the testes and expressed at much lower levels in other tissues including the kidney and the brain (Ambrose et al., 1992; Premont et al., 1996; Virlon et al., 1998; Sallese et al., 2000; Willets et al., 2003).

Human GRK4 exists as four splice variants: GRK4α, GRK4β, GRK4γ, and GRK4δ (Premont et al., 1996). GRK4α is the full-length version, most homologous with the other GRKs. GRK4β is missing the sequence encoded by exon 2, resulting in a 32-residue deletion that encompasses the phosphatidylinositol bisphosphate binding domain near the N terminus. GRK4γ is missing the sequence encoded by exon 15, resulting in a 46-residue deletion near the C terminus, and GRK4δ, the shortest variant, is missing both alternatively spliced exons. Rankin et al. (2006) determined that the D_1 receptor was constitutively phosphorylated only by coexpression with GRK4α and not with GRK2, GRK3, or any of the shorter splice variants of GRK4.

This work raises many interesting questions pertaining to the specificity of the response. First, is this a unique characteristic of the D_1 receptor, or will other GPCRs be found to be constitutively phosphorylated by GRK4? GRK4 is capable of phosphorylating and/or desensitizing activated forms of rhodopsin (Virlon et al., 1998), the follicle-stimulating hormone

receptor (Lazari et al., 1999), the m2 muscarinic receptor (Tsuga et al., 1998), the luteinizing hormone/chorionic gonadotropin receptor (Premont et al., 1996), and the β_2-adrenoceptor (Premont et al., 1996). GRK4 can also regulate the calcium-sensing receptor (Pi et al., 2005) and is the endogenous GRK that mediates homologous desensitization of two other class C GPCRs in cerebellar neurons, the mGluR1 (Sallese et al., 2000) and $GABA_B$ (Perroy et al., 2003) receptors, in addition to being an endogenous regulator of the D_1 receptor in renal proximal tubule cells (Felder et al., 2002; Watanabe et al., 2002). There is one interesting report that GRK4 causes constitutive phosphorylation of the β_2-adrenoceptor in HEK293 cells, with no additional phosphorylation induced by agonist treatment, and also causes enhanced agonist-independent internalization of the receptor (Ménard et al., 1996). It seems likely that GRK4 will be found to catalyze constitutive phosphorylation of additional GPCRs.

A second question is whether constitutive GPCR phosphorylation is restricted to the GRK4 subtype. Rankin et al. (2006) determined that the D_1 receptor is not constitutively phosphorylated by GRK2/3, the two members of the βARK subfamily. Although other members of the GRK4 subfamily were not tested by Rankin et al. (2006), previous work has shown robust agonist-stimulated phosphorylation of the D_1 receptor by GRK5 in HEK293 cells (Tiberi et al., 1996), suggesting a preference of that kinase for the activated state of the receptor. For the β_2-adrenoceptor, all three members of the GRK4 subfamily caused significantly more basal phosphorylation than GRK1–3, but only in the presence of GRK4 was there no additional agonist-induced phosphorylation (Ménard et al., 1996). A mechanistic basis for the greater propensity of members of the GRK4 subfamily to phosphorylate inactive GPCRs could be their constitutive localization at the membrane, in contrast to GRK2/3, whose translocation to the membrane is aided by free G$\beta\gamma$ produced by GPCR-activated heterotrimeric G proteins. If GRK4 is more likely than other members of that subfamily to exhibit no preference for activated GPCR over inactive receptor, an interesting line of investigation will be to identify the unique features of GRK4 that are responsible for this characteristic.

A system in which a GRK constitutively desensitizes a GPCR seems susceptible to dysregulation, in contrast to the homeostasis conferred by homologous desensitization in which only the activated receptor is desensitized, because an overabundance of the GRK or a mutation that enhances its activity, as in the kidney (see below), could cause perpetual desensitization. Is this the reason for the restricted distribution and generally low abundance of GRK4? Is there a unique characteristic of GPCR function in testes that makes it advantageous to have a high level of GRK4 and, hypothetically, constitutive desensitization?

Finally, what is the physiological relevance of the constitutive phosphorylation of the D_1 receptor by GRK4? Significant expression of GRK4 in D_1 receptor-dense brain regions has not been described. In renal proximal tubules, on the other hand, where the D_1 receptor regulates natriuresis, genetic hypertension in rats and human essential hypertension are associated with nonresponsiveness to dopamine because of constitutive desensitization of the D_1 receptor (Zeng et al., 2004). GRK4, in particular the GRK4γ splice variant, catalyzes D_1 receptor hyperphosphorylation in renal proximal tubule cells from subjects with essential hypertension (Felder et al., 2002). Essential hypertension is linked to a locus that includes GRK4 (Casari et al., 1995) and is associated with nonsynonymous single nucleotide polymorphisms in the coding region of GRK4 (Speirs et al., 2004). When heterologously expressed with the D_1 receptor in Chinese hamster ovary cells, allelic variants of GRK4γ (R65L, A142V, A486V) cause enhanced desensitization and agonist-independent phosphorylation of the receptor, and transgenic mice expressing the A142V variant of GRK4γ, but not wild-type GRK4γ, are hypertensive and lack D_1 agonist-induced diuresis and natriuresis (Felder et al., 2002). The parallels between the work of Rankin et al. (2006) and the role of GRK4 in the kidney are not exact, because GRK4γ seems to be the variant that regulates the D_1 receptor in renal proximal tubule cells, whereas in HEK293 cells, GRK4γ and its allelic variants, as well as GRK4β and GRK4δ, do not enhance basal phosphorylation of the D_1 receptor or have any effect that can be distinguished from endogenous GRKs (Rankin et al., 2006). It is possible that the lack of effect of GRK4γ in HEK293 cells can be attributed to differences in the cellular environment, and an interesting line of investigation will be to evaluate the effect of GRK4α, and the effect of the single nucleotide polymorphisms in the context of GRK4α, on D_1 receptor function in renal proximal tubule cells. Despite the discrepancies, the similarity between the observation that GRK4 does not distinguish between inactive and active D_1 receptor in HEK293 cells and the accumulating evidence that GRK4 hyperactivity is the cause of insensitivity to dopamine in essential hypertension suggests that further investigation of the mechanisms of this unusual characteristic of GRK4 will also help to elucidate fundamental mechanisms of the disorder.

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Address correspondence to: Kim A. Neve, VA Medical Center (R&D-30), 3710 SW US Veterans Hospital Rd, Portland, OR 97239-2999. E-mail: nevek@ohsu.edu