

MINIREVIEW

Functional Selectivity in Adrenergic and Angiotensin Signaling Systems

Chetan B. Patel, Nabila Noor, and Howard A. Rockman

Departments of Medicine (C.B.P., H.A.R.), Cell Biology (H.A.R.), and Molecular Genetics and Microbiology (H.A.R.), Duke University School of Medicine (N.N.), Durham, North Carolina

Received June 19, 2010; accepted September 15, 2010

ABSTRACT

β -Adrenergic and angiotensin II type 1A receptors are therapeutic targets for the treatment of a number of common human diseases. Pharmacological agents designed as antagonists for these receptors have positively affected the morbidity and mortality of patients with hypertension, heart failure, and renal disease. Antagonism of these receptors, however, may only partially explain the therapeutic benefits of β -blockers and angiotensin receptor blockers given the emerging concept of functional selectivity or biased agonism. This new pharmacological paradigm suggests that multiple signaling pathways can be differentially modified by a single ligand-receptor

interaction. This review examines the functional selectivity of β -adrenergic and angiotensin II type 1A receptors with respect to their ability to signal via both G protein-dependent and G protein-independent mechanisms, with a focus on the multifunctional protein β -arrestin. Also highlighted are the concept of “biased signaling” through β -arrestin mediated pathways, the affect of ligand/receptor modification on such biased agonism, and the implications of functional selectivity for the development of the next generation of β -blockers and angiotensin receptor blockers.

Introduction

β -adrenergic receptors (β 1AR and β 2AR) and angiotensin II type 1A receptors (AT1aR) are seven transmembrane receptors that are common therapeutic targets for the treatment of hypertension, renal disease, and heart failure. Indeed, pharmacological agents that target these receptors as antagonists (β blockers and angiotensin receptor blockers) have reduced complications and improved survival in patients with elevated blood pressure, renal impairment, and decreased cardiac function (Hjalmarson et al., 2000; Brenner et al., 2001; Dahlöf et al., 2002; Packer et al., 2002). Although these agents were developed as classic antagonists for their

respective receptors (to block deleterious effects of classic G protein signaling), the emerging concept of functional selectivity challenges the traditional paradigm of these receptors as simple on-off switches (Urban et al., 2007; Kenakin, 2001, 2005). Implicit in the traditional scheme of receptor function is the concept of linear efficacy, which is the notion that a given ligand is equally effective at stimulating or inhibiting all cellular responses regulated by a given receptor (traditional agonist or antagonist). In the case of G protein-coupled receptors (GPCRs), this paradigm entails either complete or partial activation or inhibition of G protein activity. However, investigations over the past 15 years have demonstrated that a single ligand can activate multiple signaling pathways with differing efficacies by inducing distinct conformational changes in the receptor (Kenakin, 2001). For example, a characteristically defined antagonist for a GPCR may prevent G protein signaling by placing the receptor in a conformation that does not acti-

This work was supported in part by National Institutes of Health National Heart, Lung, and Blood Institute [Grants HL075443, HL56687]; and a Duke University Medical School Stead Fellowship.

Article, publication date, and citation information can be found at <http://molpharm.aspetjournals.org>.
doi:10.1124/mol.110.067066.

ABBREVIATIONS: β AR, β -adrenergic receptor; AT1aR, angiotensin II type 1a receptor; GRK, G protein-coupled receptor kinase; ERK, extracellular-signal receptor kinase; EGFR, epidermal growth factor receptor; MAP, mitogen-activated protein; siRNA, small interfering RNA; AngII, angiotensin II; FRET, fluorescence resonance energy transfer; ARB, angiotensin receptor blocker; CaMKII, Ca^{2+} /calmodulin kinase II; PKC, protein kinase C; Epac, cAMP-dependent guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; R031-8425, 2-[8-(aminomethyl)-6,7,8,9-tetrahydropyrido[1,2-a]indol-3-yl]-3-(1-methylindol-3-yl)maleimide.

vate an associated G protein. However, the receptor conformation induced by this ligand may simultaneously permit signaling through another mechanism, unrelated to G protein activity, thus making it an agonist for this second pathway (Fig. 1). This phenomenon, described as functional selectivity, collateral/pluridimensional efficacy, or biased agonism, has major implications for pharmacological therapeutics targeting the adrenergic/angiotensin pathways (Kenakin, 2003, 2004, 2005; Urban et al., 2007). More specifically, the possibility of selecting or designing novel ligands that differentially activate only a subset of functions via a single receptor holds great promise for the treatment of diseases such as heart failure and hypertension.

Classic G-Protein Signaling and Regulation

When bound to ligands that act as agonists, GPCRs are stabilized in an active conformation and stimulate hetero-

trimeric guanine nucleotide-binding regulatory proteins (G proteins) through their intracellular domains. Interaction of the receptor with an agonist promotes signaling by modulating the activity of effector enzymes or ion channels, leading to the generation of second-messenger molecules (Rockman et al., 2002). Rapid termination of the signal, a process known as desensitization, occurs by uncoupling the receptor from its G protein through a process that generally requires phosphorylation by receptor kinases (Lefkowitz, 1998; Lefkowitz and Shenoy, 2005). The G protein-coupled receptor kinases (GRKs 1–7) preferentially phosphorylate agonist-occupied or -activated receptors and enhance the affinity of the receptor for cytosolic β -arrestins, which promote G protein uncoupling (Lefkowitz and Shenoy, 2005). Increased GRK activity and β -arrestin binding were traditionally believed to force the equilibrium of these receptors toward desensitization;

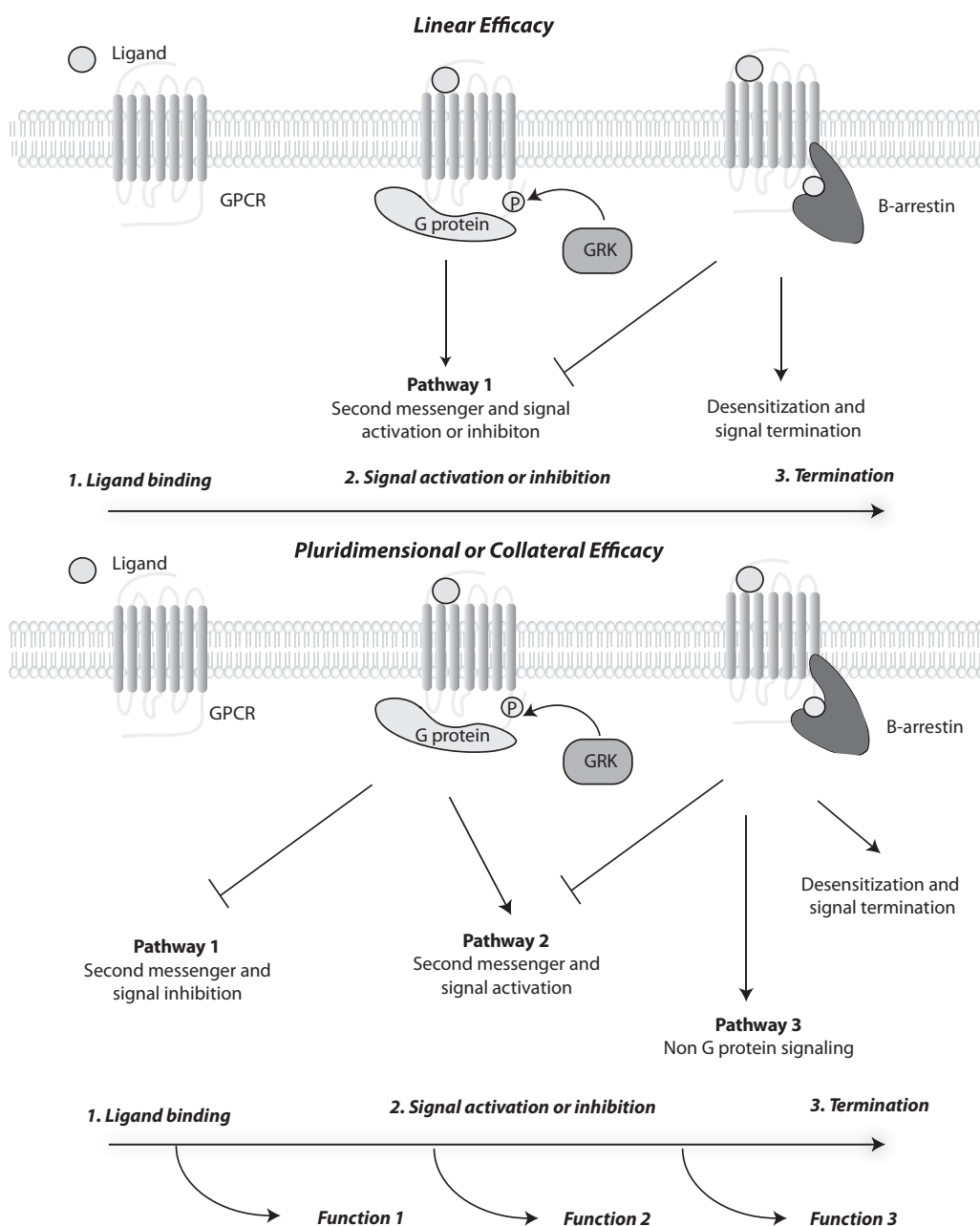


Fig. 1. Collateral efficacy in GPCR signaling. In the traditional paradigm of linear efficacy, a given agonist or antagonist for a GPCR initiates or inhibits a signaling pathway before the process is terminated by regulatory proteins such as GRKs and β -arrestins. In collateral or pluridimensional efficacy, a given ligand may be an antagonist for one pathway whereas simultaneously permitting activation of other pathways through G protein-dependent and -independent signaling. Therefore, multiple functions may be seen via a single receptor-ligand interaction and a subsequent unique receptor conformation change.

however, these proteins are now known to also actively promote cellular signaling independent of G protein activation (Lefkowitz and Shenoy, 2005; DeWire et al., 2007; Noma et al., 2007).

G Protein-Independent Signaling: Activation of the Mitogen-Activated Protein Kinases and the Role of β -Arrestin

G protein-independent signaling through GPCRs has become a major focus for investigation as the concept of functional selectivity has expanded to a number of pathways and receptor types (Kenakin, 2004; Galandrin et al., 2007). One widely studied signaling system is the mitogenic extracellular-signal receptor kinase (ERK) signaling pathway (McKay and Morrison, 2007). ERK signaling is characteristically activated by agonist stimulation of receptor tyrosine kinases and many GPCRs (Pierce et al., 2000; Rozengurt, 2007; Strachan et al., 2010), an example being the epidermal growth factor receptor (EGFR) that initiates a signaling cascade to promote mitogenic and antiapoptotic effects (Jarpe et al., 1998; McKay and Morrison, 2007). These antiapoptotic signals are mediated through both inhibition of caspase activity (Allan et al., 2003; Luciano et al., 2003) and enhancement of cellular proliferation by activation of proteins involved in nucleic acid synthesis (Graves et al., 2000), transcription (Stefanovsky et al., 2006), and translation (Waskiewicz et al., 1997). Indeed, a recent study has elucidated one of the mechanisms behind GPCR mediated antiapoptosis through ERK signaling and highlights a major role for β -arrestins as a mediator of G protein-independent signaling (Ahn et al., 2009).

G Protein Switching and MAP Kinase Signaling. The ability of the GPCRs to activate ERK signaling has been an important discovery in the evolving concept of functional selectivity by emphasizing the capacity of the receptor to function with greater complexity than as a simple on-off switch. The GPCR's role in activating MAP kinase signaling was initially attributed to components of the G protein complex such as $G\beta\gamma$ subunits of the inhibitory G_i protein (Luttrell et al., 1996; Daaka et al., 1997; Zou et al., 1998). The involvement of G_i led to the concept of "G protein switching," in which both the β_1 AR and β_2 AR were found to be able to induce ERK signaling through a switch in the coupling from G_s to G_i proteins (Daaka et al., 1997; Martin et al., 2004). This "switching" was subsequently found to be dependent on phosphorylation of the β_1 AR and β_2 AR by protein kinase A, thus providing early insight into the important role of receptor phosphorylation in activation of multiple signaling events (Daaka et al., 1997; Martin et al., 2004). These studies demonstrated that a mechanism shown previously to mediate uncoupling of the β AR from G_s (and decrease signaling) also serves to "switch" coupling of this receptor from G_s to G_i and initiate a new set of signaling events (Daaka et al., 1997; Zhu et al., 2001; Martin et al., 2004).

β -Arrestin-Mediated MAP Kinase Signaling. Although G protein-switching provided initial insight into GPCR-mediated activation of the MAP kinase system, the discovery that β -arrestin could play a major role in ERK signaling provided further detail into both the complex nature of GPCR signaling and the multifunctional roles of the β -arrestin molecule (Maudsley et al., 2000). β -Arrestin-mediated ERK signaling has been

shown for both β ARs and the AT1aR with distinct kinetics and molecular consequences. For example, in cells overexpressing the AT1aR or the β_2 AR, G protein activation through the ligand-bound receptor leads to peak ERK activity within 2 to 5 min (Ahn et al., 2004; Shenoy et al., 2006). In contrast, β -arrestin-mediated signaling after angiotensin II (AngII) stimulation of AT1aR or isoproterenol stimulation of β_2 AR has a slower and more prolonged pattern of ERK activation (Ahn et al., 2004; Shenoy et al., 2006) (Fig. 2). Furthermore, β -arrestin-mediated signaling by the AT1aR promotes only cytoplasmic ERK localization, whereas G protein-mediated ERK activation results in both nuclear and cytoplasmic localization (Gáborik et al., 2003; Tohgo et al., 2003; Ahn et al., 2004).

The process described previously of GRK phosphorylation of the receptor is a critical step in directing this alternative β -arrestin-mediated pathway, and detailed investigations highlight the different roles of the various GRK isoforms (Violin et al., 2006). Inhibition of GRK 5 or 6 using siRNA technology attenuates β -arrestin-mediated ERK activation via the β_1 AR and AT1aR, whereas it remains unaffected (or may even increase) when siRNA is used to knockdown GRK 2 or 3 expression (Kim et al., 2005, 2008; Noma et al., 2007). β -Arrestin-dependent ERK signaling is also augmented by the ligand-bound AT1aR or β_2 AR when GRK 5 or 6 is overexpressed in the cell (Kim et al., 2005; Shenoy et al., 2006). Remarkably, the β -arrestin component of the ERK response is reciprocally diminished by GRK 2 overexpression (Kim et al., 2005; Shenoy et al., 2006). Investigation of β -arrestin-mediated signaling via the β_1 AR has not only confirmed findings with other receptors but has also led to the discovery of EGFR transactivation as a mechanism for β -arrestin-mediated ERK signaling (Noma et al., 2007). Indeed, β_1 AR-stimulated β -arrestin-dependent ERK signaling requires GRK 5/6 and results in the extracellular release of membrane-bound heparin-binding epidermal growth factor-like

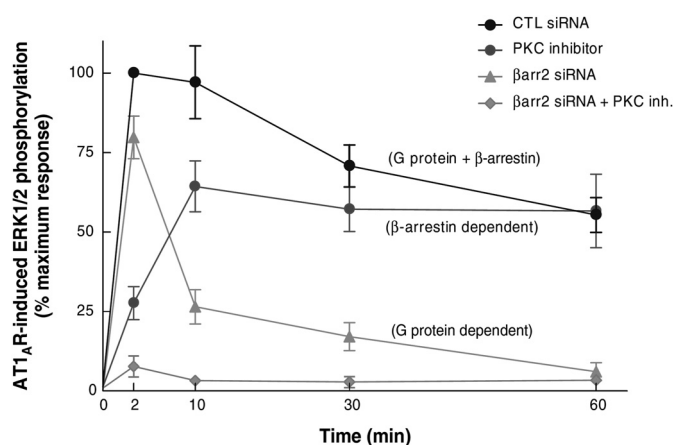


Fig. 2. Time course of β -arrestin and G protein-mediated ERK signaling. In cells stably expressing the AT1aR-selective inhibition of β -arrestin and G protein pathways highlight temporal differences in MAP kinase signaling through different mediators via the same receptor. Cells were transfected with control siRNA or β -arrestin2 siRNA for 72 h and treated with either PKC inhibitor or dimethyl sulfoxide vehicle for 20 min before stimulation. Graph is plotted from five individual experiments as percentage of maximal ERK activation at 2 min in the control siRNA, dimethyl sulfoxide-treated sample. [Reproduced from Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S, Reiter E, Premont RT, Lichtarge O, and Lefkowitz RJ (2006) Beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor. *J Biol Chem* 281:1261–1273. Copyright © 2006 American Society for Biochemistry and Molecular Biology. Used with permission.]

growth factor to transactivate EGFR (Noma et al., 2007). It is noteworthy that a β -arrestin-dependent mechanism for transactivation of EGFR by AT1aRs, has been reported recently after activation of endogenously expressed angiotensin receptors in vascular smooth muscle cells (Kim et al., 2009).

Ligand-Directed Signaling at Adrenergic and Angiotensin Receptors

As the pluridimensional efficacy of receptors in the β -adrenergic and angiotensin receptor signaling pathways has been dissected, interest has grown regarding the possibility of selectively activating the different pathways with currently available or novel ligands. The functional selectivity of different ligands has been evaluated by screening a variety of compounds for the β -adrenergic receptors. Baker et al. (2003) showed the agonist and inverse agonist (defined as a decrease in basal or constitutive activity after ligand binding) response to β 2AR activation with a series of ligands using isoproterenol as a reference compound. Despite a significant decrease in cAMP production (inverse agonist effect), certain ligands (in particular the traditional β AR antagonist propranolol) demonstrate increased cAMP response element-mediated transcription and MAP kinase activation (Baker et al., 2003). In a similar set of experiments involving the stimulation of β 1AR and β 2AR with a variety of traditional antagonists, Galandrin and Bouvier (2006) identified complex "efficacy profiles" for both receptor types. For the β 1AR, carvedilol, bucindolol, and labetalol activate both adenylyl cyclase and ERK pathways to varying degrees, with carvedilol having the greatest ERK signaling among traditional β -blockers (Galandrin and Bouvier, 2006). For the β 2AR, the same three agents demonstrate both cAMP and ERK activity but with variable efficacy. Although these initial screening studies did not directly evaluate the mechanism of ERK activation and role of β -arrestin as a critical signaling molecule in the activation of the ERK pathway, more recent investigations have examined the ability of ligands to recruit β -arrestin and activate ERK in a " β -arrestin-biased" fashion (Wisler et al., 2007; Kim et al., 2008).

β -Arrestin Bias and Ligand-Directed Signaling through GPCRs. Using fluorescence resonance energy transfer (FRET) to quantify the magnitude of β -arrestin recruitment to β 2ARs, a number of traditional β 2AR agonists were found to exhibit variable efficacies for β -arrestin recruitment to the receptor (Drake et al., 2008). By comparing the ratio of cAMP generation with the rate and magnitude of β -arrestin recruitment to the β 2AR, Drake et al. (2008) were able to calculate a ligand-specific β -arrestin "bias factor." Based on this bias factor, three of the ligands, ethyl-norepinephrine, isoeutharine, and *n*-cyclopentylbutanephine, show the greatest β -arrestin bias (Fig. 3). In a related study, a series of traditional β AR antagonists were also evaluated for relative G protein and β -arrestin-mediated signaling (Wisler et al., 2007). Of 17 ligands tested, only carvedilol demonstrated a unique signaling profile of negative efficacy for G_s -dependent adenylyl cyclase activation, although still weakly promoting β -arrestin recruitment, internalization, and activation of ERK signaling. Carvedilol and alprenolol are also able to promote β 1AR-mediated EGFR transactivation that occurs through the recruitment and activation of β -arrestin as shown in both in cell culture and murine myocardium (Kim

et al., 2008) (Fig. 4), suggesting that this could be a primary mechanism for β 1AR ligand-induced ERK activation in the heart (Noma et al., 2007; Kim et al., 2008). Although these data suggest that measuring different signaling events downstream of a single ligand-receptor interaction can give clues to the degree of selective activation, it is important to note that the optimal means for quantifying ligand-directed "bias" for a GPCR ligand is undetermined, but remains an active field of research in receptor pharmacology (Kenakin, 2005, 2009; Kenakin and Miller, 2010; Rajagopal et al., 2010). A major challenge is the parallel activation of a signaling pathway by multiple upstream effectors and interpreting this signaling product as a measure of functional selectivity. An example is MAP kinase signaling that can be activated by G protein, Src activation, and β -arrestin, although the relative contribution may be different based on the ligand used for activation. As the field moves forward,

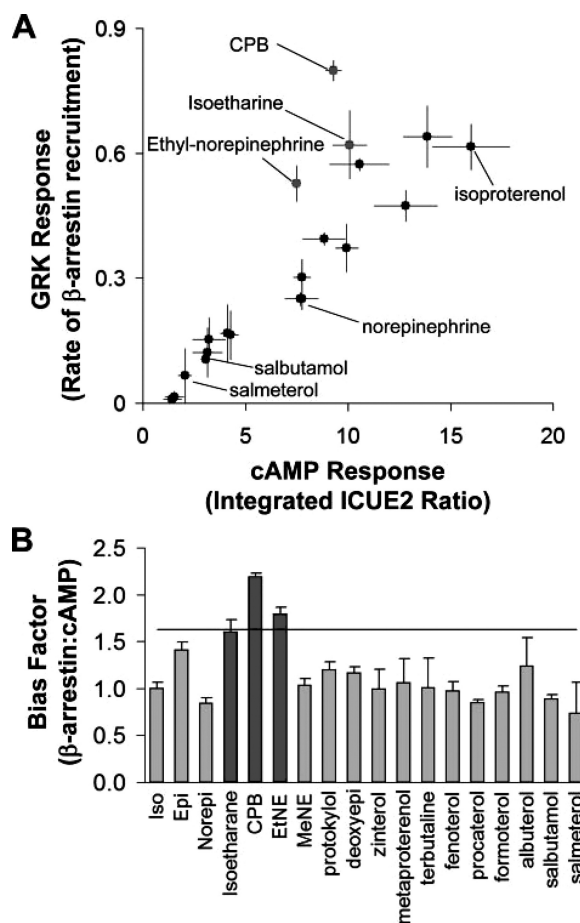


Fig. 3. Identifying β -arrestin-biased ligands. Using FRET-based assays, Drake et al. (2008) identified ligands for the β 2AR that were able to activate G protein activity and recruit β -arrestin in a robust fashion. By calculating the ratio of β -arrestin recruitment to cAMP generation, they defined ligand-specific bias factors. cAMP generation was measured using a modified Epac molecule in a FRET-based assay (indicator of cAMP using Epac). Upon binding cAMP, this Epac molecule changes conformation, resulting in a loss of basal FRET signal. GRK activity was derived from β -arrestin recruitment to the receptor by measuring the FRET signal generated when yellow fluorescent protein-tagged β -arrestin interacted with cyan fluorescent protein-tagged receptor. [Reproduced from Drake MT, Violin JD, Whalen EJ, Wisler JW, Shenoy SK, and Lefkowitz RJ (2008) β -arrestin-biased agonism at the β 2-adrenergic receptor. *J Biol Chem* 283:5669–5676. Copyright © 2008 American Society for Biochemistry and Molecular Biology. Used with permission.]

experimental evidence will have to show ligand-specific efficacy profiles for multiple measures, including intermediate proteins upstream of an effector protein such as MAP kinase.

Conformational Changes in GPCRs and Ligand-Directed Signaling

Current concepts support the notion that ligands such as carvedilol can induce unique conformations within the β AR that decrease G protein coupling while promoting selective GRK phosphorylation and β -arrestin-mediated signaling. In fact, ligand-induced changes in receptor conformation have been directly studied using a modified β 2AR with fluorophores ligated to the C terminus and to a cysteine residue in the sixth transmembrane region (Granier et al., 2007). Using this reporter, the Kobilka group has shown that different ligands induce variable changes in receptor conformation involving the C terminus (Granier et al., 2007). More specifically, ligands known to activate β -arrestin-mediated MAP kinase signaling (i.e., isoproterenol and epinephrine) seem to

induce a unique conformation at the proximal C-terminal tail compared with ligands that do not mediate β -arrestin-mediated signaling (Ghanouni et al., 2001; Granier et al., 2007).

The AT1aR has also been shown to be subject to ligand-directed signaling by using an analog of AngII known as SII AngII (Wei et al., 2003). SII is a synthetically generated compound that does not induce G protein activity when bound to the AT1aR but maintains the capacity to recruit β -arrestin. Therefore, binding of SII to the receptor results in no G protein-mediated protein kinase C (PKC) activation but a robust β -arrestin-dependent ERK signaling response (Wei et al., 2003; Ahn et al., 2004). In the heart, treatment with SII leads to ERK signal transduction and enhanced myocyte contractility (Rajagopal et al., 2006; Aplin et al., 2007). Unlike β -blockers, however, none of the currently available angiotensin receptor blockers (ARBs) have demonstrated bias (R. J. Lefkowitz, unpublished data). It is noteworthy that recent investigation has shown that membrane stretch can induce β -arrestin-biased signaling of the AT1aR in the ab-

EGFR-GFP in WT β 1AR

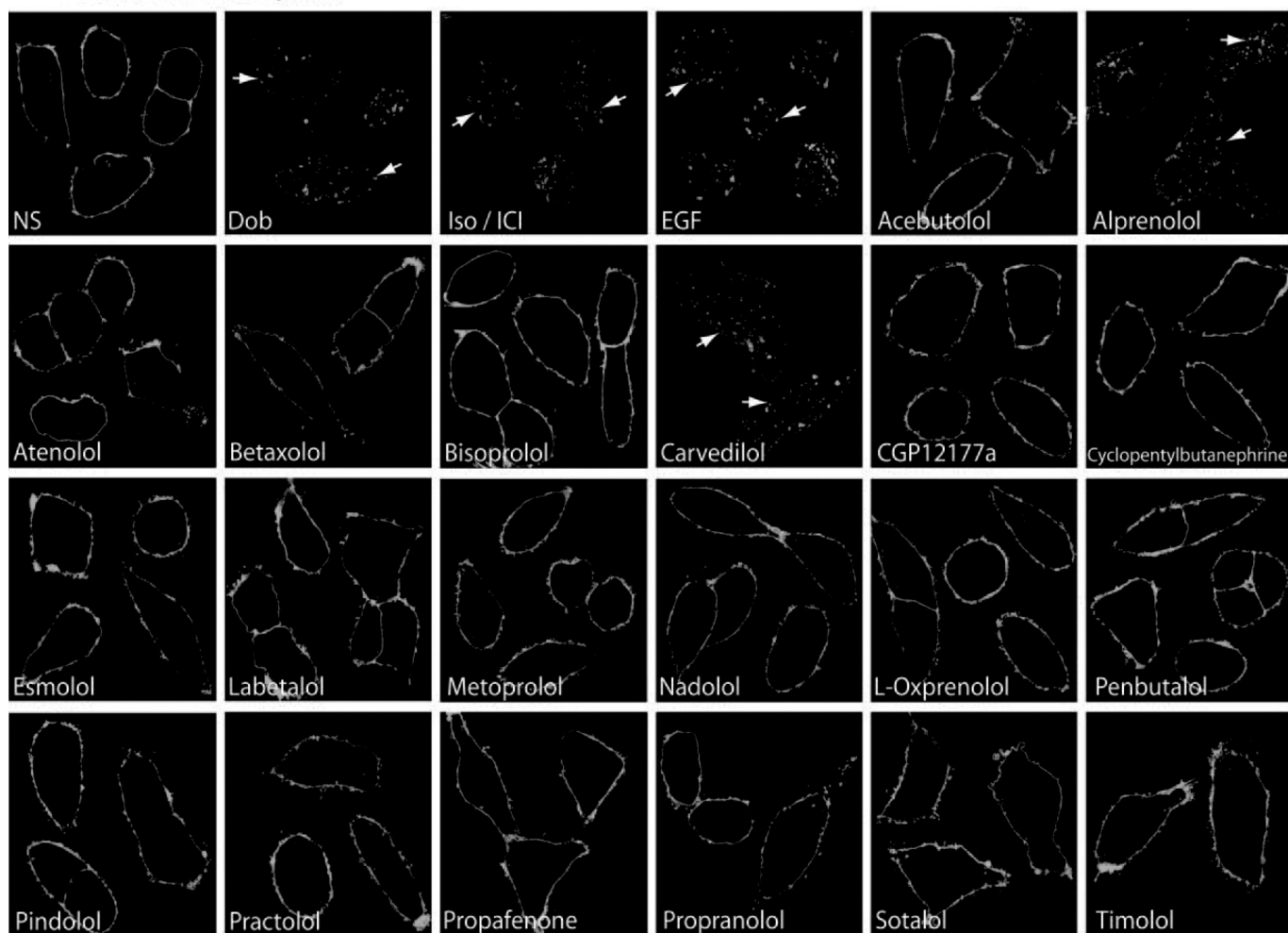


Fig. 4. Carvedilol and alprenolol stimulate EGFR transactivation. After screening more than 20 classic antagonists for the β 1AR, only carvedilol and alprenolol were able to induce internalization of the EGFR in a manner similar to agonist stimulation of the β 1AR or direct activation of EGFR (white arrows). This process of transactivation was found to be β -arrestin dependent and highlights the need to evaluate the modulation of other potentially beneficial pathways by classic antagonists of the β ARs and AT1AR. Experiments shown were performed in human embryonic kidney 293 cells. [Adapted from Kim IM, Tilley DG, Chen J, Salazar NC, Whalen EJ, Violin JD, and Rockman HA (2008) Beta-blockers alprenolol and carvedilol stimulate beta-arrestin-mediated EGFR transactivation. *Proc Natl Acad Sci USA* 105:14555–14560. Copyright © 2008 National Academy of Sciences, U.S.A. Used with permission.]

sence of ligand (Rakesh et al., 2010). Both in cell culture and an ex vivo murine heart model, mechanical stretch of a cell overexpressing the AT1aR resulted in ERK activation. This response was inhibited by β -arrestin knockdown in the cell or repeating the experiment in hearts from β -arrestin knockout mice. These findings were exclusive to the AT1aR (and not seen with the β 1ARs) and suggest that this receptor can adopt a conformation when stretched that promotes β -arrestin-mediated signaling. Further investigation into the conformational changes induced by agents such as SII and mechanical stretch of the receptor may facilitate the development of biased AT1aR ligands.

Receptor-Mediated Functional Selectivity at Adrenergic Receptors and Angiotensin Receptors

With evidence accumulating that ligand-induced receptor conformation plays a pivotal role in signaling, receptors with structural modifications that activate only certain pathways provide a valuable tool for elucidating the mechanisms that account for pluridimensional efficacy. Shenoy and others used evolutionary trace analysis to rationally modify the β 2AR amino acid residues at positions 68 (threonine), 132 (tyrosine), and 219 (tyrosine) to generate a mutant receptor known as β 2AR TYY (Shenoy et al., 2006). Cellular studies using β 2AR TYY demonstrate uncoupling of the receptor from its G protein and subsequently no cAMP production upon stimulation (Shenoy et al., 2006). Remarkably, this genetically engineered receptor maintains the ability to recruit β -arrestin and induce ERK signaling but with modified kinetics compared with the wild-type β 2AR. The increase in phosphorylated ERK seems to peak later than that seen upon stimulation of wild-type β 2ARs, is absolutely sensitive to siRNA targeting β -arrestin, and is enhanced with GRK 5/6 overexpression. These findings suggest further that activation of β -arrestin-mediated signaling requires specific GRKs to modify the receptor and promote signaling.

Mutations in the AT1 receptor by modification of the conserved sequence Asp125-Arg126-Tyr127 to Ala125-Ala126-Tyr (Gáborik et al., 2003) have also been engineered and lead to a receptor (DRY/AAY) with abolished G protein signaling that is also still able to recruit β -arrestin and mediate ERK signaling (Wei et al., 2003). ERK signaling is not sensitive to the PKC inhibitor 2-[8-(aminomethyl)-6,7,8,9-tetrahydropyrido[1,2-a]indol-3-yl]-3-(1-methylindol-3-yl)maleimide (R031-8425) but is attenuated by siRNA-mediated β -arrestin knockdown. Consistent with prior data regarding β -arrestin-mediated ERK activation, activation of this receptor does not lead to nuclear localization of ERK (Ahn et al., 2004) or an increase in transcriptional activity (Gáborik et al., 2003).

Although a naturally occurring or engineered β 1AR mutant that displays similar activity has not yet been described, another β 1AR mutant (GRK⁻ β 1AR) that has disrupted GRK phosphorylation sites and is therefore unable to recruit β -arrestin has proven useful in understanding β -arrestin-mediated ERK signaling in the heart (Noma et al., 2007). Indeed, transgenic mice overexpressing the β 1AR mutant GRK⁻ β 1AR, which cannot transactivate EGFRs, develop worse cardiac dysfunction and increased myocyte apoptosis in response to long-term catecholamine stimulation. In addition to implicating EGFR transactivation as a protective mecha-

nism in the development of heart failure, this study emphasizes the importance of receptor-based functional selectivity in modifying disease states and provides impetus for developing novel therapeutics that selectively activate antiapoptotic signaling.

Physiological Implications of Biased Agonists for the GPCRs. The discovery and development of biased agonists for the β ARs and AT1aR seems possible given the encouraging data with β -arrestin-biased receptors and ligands described above. However, we are only at the preliminary stages of fully understanding the complex nature of β -arrestin as an adaptor protein for cellular signaling. In addition, it will be important to examine other non- β -arrestin, non-G protein-mediated signaling pathways that are differentially influenced by GPCR ligands with respect to important physiological outcomes and adverse effect profiles. For example, transgenic mice with cardiac-specific expression of an AT1aR mutant containing a modified second intracellular loop (leading to G protein uncoupling) show increased hypertrophy and decreased cardiac function in response to long-term AngII infusion compared with animals overexpressing the wild-type AT1aR (Zhai et al., 2005). Upon histological analysis, the wild-type AT1aR animals demonstrate increased fibrosis and apoptosis compared with mice expressing the mutant receptor, suggesting a progrowth bias driven by the mutant receptor (Zhai et al., 2005). These data highlight the importance of considering the balance and regulation of different signaling pathways downstream of a GPCR.

β -Arrestin: a Scaffold for Functional Selectivity in Adrenergic and Angiotensin Signaling

In the preceding sections, we have described the complex interplay between receptor structure, ligand binding, receptor phosphorylation, and conformational changes that underlie the functional selectivity of the β 1AR, β 2AR, and AT1aR. In the model described above, selective changes in receptor conformation induced by GRK-specific phosphorylation lead to variable efficacy of β -arrestin recruitment to the receptor and a resultant change in β -arrestin-mediated signaling (Shukla et al., 2008). Although this model changes the traditional paradigm of receptor signaling, recent evidence highlights another layer of complexity at the level of the multifunctional β -arrestin protein. With the emerging concept of this protein as a scaffold for other enzymes, Shukla and others have evaluated conformational changes in the β -arrestin molecule using a novel intramolecular biosensor (Charest et al., 2005; Shukla et al., 2008). In studies using the biased AT1aR ligand SII, Shukla et al. (2008) show that SII induces conformational changes in the β -arrestin molecule that differ from those induced by angiotensin or the traditional ARB valsartan. The conformational change induced by SII (measured as a change in orientation between the C and N termini of β -arrestin) is similar to that observed with the β -arrestin biased receptors β 2AR TYY and AT1aR DRY/AAY (Shukla et al., 2008) upon stimulation with ISO and AngII, respectively. Although the impact of selective GRK-mediated receptor phosphorylation on β -arrestin conformation has not been directly studied, the sites of receptor phosphorylation may have important implications for the

ultimate conformation adopted by this protein and for cellular signaling. For example, ligand-dependent effects of the receptor mediated by GRKs 5 and 6 seem to be associated with physiological consequences different from those mediated by GRK 2 (Kim et al., 2005; Noma et al., 2007). The conformations adopted by β -arrestin after phosphorylation of a given receptor (i.e., β 1AR) by a specific GRK (i.e., GRK 5) have yet to be fully defined, but these may be key determinants in propagating non-G protein-mediated signaling via the GPCRs.

β -Arrestin as a Multifunctional Regulator of GPCR Signaling. There is mounting evidence that β -arrestin conformation is an important regulatory factor in determining the proteins that the molecule recruits. One such example is a recent study by Mangmool et al. (2010), which shows that activation of the β 1AR induces a conformational change in β -arrestin that promotes a stable complex between β -arrestin, Ca^{2+} /calmodulin kinase II (CaMKII) and the cAMP-dependent guanine-nucleotide exchange factor (Epac). The association of β -arrestin with the β 1AR stabilizes a β -arrestin-CaMKII-Epac complex and promotes CaMKII signaling (Mangmool et al., 2010). It is noteworthy that this β -arrestin conformation and stable complex are not formed after activation of the β 2AR, despite an equivalent amount of β -arrestin recruitment to both β ARs. Moreover, by using β AR chimeras (i.e., β 2AR with β 1AR C-terminal tail), it was shown that the C terminus of the β 1AR contains structural elements that promote β -arrestin mediated CaMKII signaling (Mangmool et al., 2010). In this case, β -arrestin provides a

scaffold for CaMKII signaling by effectively bridging G protein signaling (cAMP activation of Epac) with CaMKII to promote this cascade after β 1AR stimulation (Fig. 5).

The multifunctional roles of β -arrestin also seem to have an impact beyond the level of the receptor. For example, using a mass spectrometry-based proteomics approach, Xiao et al. (2007) have discovered a multitude of proteins interacting with β -arrestin in the basal state and after stimulation of the AT1aR. Of the greater than 300 proteins found to interact with β -arrestin, proteins involved in cellular signaling such as kinases, phosphatases and nucleic acid binding proteins are highly represented in the " β -arrestin interactome." This study underscores the pivotal role of β -arrestin in cellular physiology, and future research will undoubtedly examine differences in the interactome upon activation of β -arrestin-biased receptors and ligands.

Polymorphic Variation in Adrenergic and Angiotensin Receptors

As data continue to accumulate regarding the functional selectivity of the angiotensin and adrenergic receptors, interest has grown in the pluridimensional efficacy of naturally occurring human receptor mutants. Indeed, a better understanding of the selectivity of these mutants for a particular signaling pathway could lead to unique functional selectivity profiles for different ligands, which could lead to differential drug selection based on a patient's genotype. For example, a ligand for the β 1AR may induce greater biased- β -arrestin

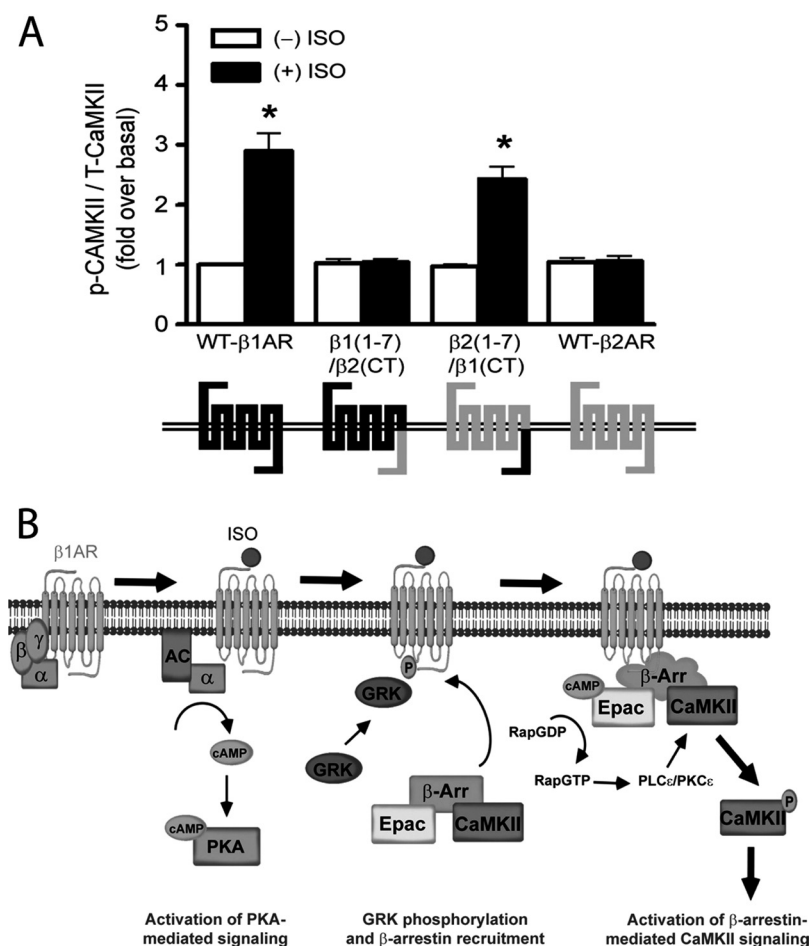


Fig. 5. β -Arrestin scaffolds CaMKII. A, using β AR chimeras (i.e., β 2AR with β 1AR C-terminal tail), Mangmool et al. (2010) show that the c-terminal of the β 1AR is the key regulatory component that promotes β -arrestin mediated CaMKII signaling upon ligand stimulation. B, a schematic representation for β -arrestin as a stable scaffold for CaMKII signaling by effectively recruiting Epac and CaMKII to promote this signaling cascade at the β 1AR. The unique conformation adopted by β -arrestin upon interaction with GPCRs and the subsequent downstream events warrant further study [Reproduced from Mangmool S, Shukla AK, and Rockman HA (2010) beta-Arrestin-dependent activation of Ca^{2+} /calmodulin kinase II after beta(1)-adrenergic receptor stimulation. *J Cell Biol* 189:573–587. doi:10.1083/jcb.200911047 Copyright © 2010 Rockefeller University Press. Used with permission.]

signaling for one β 1AR genetic variant compared with another, potentially leading to different clinical efficacies.

Using a FRET-based biosensor, one study has demonstrated in vitro variability in the response of human β 1ARs to agonist stimulation and different inverse agonists (Rochais et al., 2007). The R389G polymorphism of the human β 1AR lies in the putative G_s binding region and may regulate G protein-mediated signaling (Mialet Perez et al., 2003). In this study, the Arg389 variant demonstrated greater sensitivity to carvedilol than the Gly389 variant with regard to inhibition of G protein signaling, a difference not seen with other inverse agonists. The Arg389 variant has also been implicated in decreased infarct size in an experimental model of myocardial infarction (Akhter et al., 2006), possibly through an increase in MAP kinase signaling. Because this polymorphism is a common variant, it has been examined in population-based studies and post hoc analyses of randomized controlled trials of β -blockers. Despite these analyses, which also include other common polymorphisms such as the serine to glycine variant at position 49 in the β 1AR (S49G) and β 2AR variants (G16R and Q27E), no consensus has been reached regarding the effects of these mutations on the natural history of disease or pharmacogenomic interactions (Lanfear et al., 2005; Shin et al., 2007; Sehnert et al., 2008).

Others have examined the role of sequence variations in GRKs in human heart failure. A polymorphism in GRK 5 (GRK5-Leu41) resulted in more uncoupling of G protein activation than the GRK 5-Gln41 variant and protected against catecholamine-induced cardiomyopathy in transgenic mice (Liggett et al., 2008). In a human association study reported in the same article, carriers of this polymorphism who have heart failure seem to have lower mortality and greater freedom from cardiac transplantation. Similar evaluation has been performed with the human AT1aR polymorphism A1166C, which demonstrates increased vasoconstrictor activity in patients with hypertension (Amant et al., 1997; Spiering et al., 2000). Only one small pilot study has demonstrated a beneficial interaction between the ARB candesartan and this polymorphism (de Deus et al., 2008). However, no rigorous in vitro or in vivo evaluation of these polymorphisms has included an assessment of G protein-independent MAP kinase signaling.

Future Challenges for Defining Functional Selectivity of Ligands

As progress is made using the currently available data to develop and test biased ligands for the β ARs and AT1aR, we anticipate a number of challenges in defining functional selectivity. As evidenced in the preceding sections and summarized in a recent article by Rajagopal et al. (2010), multiple assays have been used to define relative G protein and β -arrestin activation. Although the development of biased ligands will certainly begin at the biochemical and cellular level, we must also work to define which assays will translate into physiological effects. Factors that may play a role in differences between experimental systems and animal/human subjects include ligand concentration, receptor expression level, and the activity/expression of certain effector proteins (i.e., variable GRK isoform expression in target tissues).

Furthermore, it is important to remember that a single receptor-ligand combination can activate a downstream ef-

fector using multiple pathways and activate signaling pathways not identified by the assay being used. Therefore, the relative contribution of these multiple signaling pathways will have to be quantified before the functional selectivity of a ligand can be confidently defined. In addition to assessing intermediate proteins in different signaling cascades, it is likely investigators will rely on computational systems biology techniques to predict in quantitative manner the full complement of proteins activated by various ligands. This has been accomplished recently for the AT1aR, in which unique phosphoproteins were identified after activation by the β -arrestin biased ligand SII that were not seen with ATII stimulation (Christensen et al., 2010; Xiao et al., 2010). Computational analysis of these mass spectrometry-derived data revealed multiple novel pathways that could be activated via β -arrestin (Xiao et al., 2007). Use of such technology will likely contribute to and expedite the development of biased ligands.

Summary

The emerging concept of functional selectivity has led to exciting new discoveries about the signaling pathways regulated by GPCRs. Of particular interest has been the finding that GPCR signaling can interact with other cellular pathways via mechanisms that were once believed to desensitize the receptors and inhibit downstream activity. GRKs and the multifunctional β -arrestins seem to be critical components of the interaction between GPCRs (such as β ARs and AT1aR) and other signaling cascades such as the MAP kinase pathway. Because β ARs and AT1aRs are targets for therapeutics, there is growing interest in the development of the next generation of drugs to selectively antagonize maladaptive signaling while simultaneously augmenting beneficial pathways. In addition, naturally occurring mutations of these receptors/kinases may demonstrate varying efficacy toward different pathways. This phenomenon has major implications for future targeted therapy with existing and novel drugs directed at the adrenergic and angiotensin receptors.

Acknowledgments

We acknowledge Dr. Robert J. Lefkowitz for scientific insight, kind mentorship, and thoughtful review of this manuscript. Dr. Lefkowitz's extensive contributions to the field of seven transmembrane receptor research have made such a comprehensive review possible, and we are grateful for his comments and guidance.

References

- Ahn S, Kim J, Hara MR, Ren XR, and Lefkowitz RJ (2009) β -Arrestin-2 mediates anti-apoptotic signaling through regulation of BAD phosphorylation. *J Biol Chem* **284**:8855–8865.
- Ahn S, Shenoy SK, Wei H, and Lefkowitz RJ (2004) Differential kinetic and spatial patterns of β -arrestin and G protein-mediated ERK activation by the angiotensin II receptor. *J Biol Chem* **279**:35518–35525.
- Akhter SA, D'Souza KM, Petrashevskaya NN, Mialet-Perez J, and Liggett SB (2006) Myocardial β 1-adrenergic receptor polymorphisms affect functional recovery after ischemic injury. *Am J Physiol Heart Circ Physiol* **290**:H1427–H1432.
- Allan LA, Morrice N, Brady S, Magee G, Pathak S, and Clarke PR (2003) Inhibition of caspase-9 through phosphorylation at Thr 125 by ERK MAPK. *Nat Cell Biol* **5**:647–654.
- Amant C, Hamon M, Bateurs C, Richard F, Helbecque N, McFadden EP, Escudero X, Lablanche JM, Amouyel P, and Bertrand ME (1997) The angiotensin II type 1 receptor gene polymorphism is associated with coronary artery vasoconstriction. *J Am Coll Cardiol* **29**:486–490.
- Aplin M, Christensen GL, Schneider M, Heydorn A, Gammeltoft S, Kjølbye AL, Sheikh SP, and Hansen JL (2007) The angiotensin type 1 receptor activates extracellular signal-regulated kinases 1 and 2 by G protein-dependent and -independent pathways in cardiac myocytes and langendorff-perfused hearts. *Basic Clin Pharmacol Toxicol* **100**:289–295.

- Baker JG, Hall IP, and Hill SJ (2003) Agonist and inverse agonist actions of beta-blockers at the human beta 2-adrenoceptor provide evidence for agonist-directed signaling. *Mol Pharmacol* **64**:1357–1369.
- Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S, et al. (2001) Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* **345**:861–869.
- Charest PG, Terrillon S, and Bouvier M (2005) Monitoring agonist-promoted conformational changes of beta-arrestin in living cells by intramolecular BRET. *EMBO Rep* **6**:334–340.
- Christensen GL, Kelstrup CD, Lyngsø C, Sarwar U, Bøgebo R, Sheikh SP, Gammeltoft S, Olsen JV, and Hansen JL (2010) Quantitative phosphoproteomics dissection of seven-transmembrane receptor signaling using full and biased agonists. *Mol Cell Proteomics* **9**:1540–1553.
- Daaka Y, Luttrell LM, and Lefkowitz RJ (1997) Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* **390**:88–91.
- Dahlöf B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, Fyhrquist F, Ibsen H, Kristiansson K, Lederballe-Pedersen O, et al. (2002) Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* **359**:995–1003.
- de Denus S, Zakrzewski-Jakubiak M, Dubé MP, Bélanger F, Lepage S, Leblanc MH, Gossard D, Ducharme A, Racine N, Whittom L, et al. (2008) Effects of AGTR1 A1166C gene polymorphism in patients with heart failure treated with candesartan. *Ann Pharmacother* **42**:925–932.
- DeWire SM, Ahn S, Lefkowitz RJ, and Shenoy SK (2007) Beta-arrestins and cell signaling. *Annu Rev Physiol* **69**:483–510.
- Drake MT, Violin JD, Whalen EJ, Wisler JW, Shenoy SK, and Lefkowitz RJ (2008) beta-arrestin-biased agonism at the beta2-adrenergic receptor. *J Biol Chem* **283**:5669–5676.
- Gáborik Z, Jagadeesh G, Zhang M, Spät A, Catt KJ, and Hunyady L (2003) The role of a conserved region of the second intracellular loop in AT1 angiotensin receptor activation and signaling. *Endocrinology* **144**:2220–2228.
- Galandrin S and Bouvier M (2006) Distinct signaling profiles of beta1 and beta2 adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy. *Mol Pharmacol* **70**:1575–1584.
- Galandrin S, Oligny-Longpré G, and Bouvier M (2007) The evasive nature of drug efficacy: implications for drug discovery. *Trends Pharmacol Sci* **28**:423–430.
- Ghanouni P, Gryczynski Z, Steenhuis JJ, Lee TW, Fahrens DL, Lakowicz JR, and Kobilka BK (2001) Functionally different agonists induce distinct conformations in the G protein coupling domain of the beta 2 adrenergic receptor. *J Biol Chem* **276**:24433–24436.
- Granier S, Kim S, Shafer AM, Ratnala VR, Fung JJ, Zare RN, and Kobilka B (2007) Structure and conformational changes in the C-terminal domain of the beta2-adrenoceptor: insights from fluorescence resonance energy transfer studies. *J Biol Chem* **282**:13895–13905.
- Graves LM, Guy HI, Kozlowski P, Huang M, Lazarowski E, Pope RM, Collins MA, Dahlstrand EN, Earp HS 3rd, and Evans DR (2000) Regulation of carbamoyl phosphate synthetase by MAP kinase. *Nature* **403**:328–332.
- Hjalmarson A, Goldstein A, Bergberg B, Wedel H, Waagstein F, Kjekshus J, Wikstrand J, El Allaf D, Vitovec J, Aldershvile J, et al. (2000) Effects of controlled-release metoprolol on total mortality, hospitalizations, and well-being in patients with heart failure: the Metoprolol CR/XL Randomized Intervention Trial in congestive heart failure (MERIT-HF). MERIT-HF Study Group. *JAMA* **283**:1295–1302.
- Jarpe MB, Widmann C, Knall C, Schlesinger TK, Gibson S, Yujiri T, Fanger GR, Gelfand EW, and Johnson GL (1998) Anti-apoptotic versus pro-apoptotic signal transduction: checkpoints and stop signs along the road to death. *Oncogene* **17**:1475–1482.
- Kenakin T (2001) Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB J* **15**:598–611.
- Kenakin T (2003) Ligand-selective receptor conformations revisited: the promise and the problem. *Trends Pharmacol Sci* **24**:346–354.
- Kenakin T (2004) Principles: receptor theory in pharmacology. *Trends Pharmacol Sci* **25**:186–192.
- Kenakin T (2005) New concepts in drug discovery: collateral efficacy and permissive antagonism. *Nat Rev Drug Discov* **4**:919–927.
- Kenakin T and Miller LJ (2010) Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. *Pharmacol Rev* **62**:265–304.
- Kenakin TP (2009) 7TM receptor allostery: putting numbers to shapeshifting proteins. *Trends Pharmacol Sci* **30**:460–469.
- Kim IM, Tilley DG, Chen J, Salazar NC, Whalen EJ, Violin JD, and Rockman HA (2008) Beta-blockers alprenolol and carvedilol stimulate beta-arrestin-mediated EGFR transactivation. *Proc Natl Acad Sci USA* **105**:14555–14560.
- Kim J, Ahn S, Rajagopal K, and Lefkowitz RJ (2009) Independent beta-arrestin2 and Gq/protein kinase Czeta pathways for ERK stimulated by angiotensin type 1A receptors in vascular smooth muscle cells converge on transactivation of the epidermal growth factor receptor. *J Biol Chem* **284**:11953–11962.
- Kim J, Ahn S, Ren XR, Whalen EJ, Reiter E, Wei H, and Lefkowitz RJ (2005) Functional antagonism of different G protein-coupled receptor kinases for beta-arrestin-mediated angiotensin II receptor signaling. *Proc Natl Acad Sci USA* **102**:1442–1447.
- Lanfang DE, Jones PG, Marsh S, Cresci S, McLeod HL, and Spertus JA (2005) Beta2-adrenergic receptor genotype and survival among patients receiving beta-blocker therapy after an acute coronary syndrome. *JAMA* **294**:1526–1533.
- Lefkowitz RJ (1998) G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. *J Biol Chem* **273**:18677–18680.
- Lefkowitz RJ and Shenoy SK (2005) Transduction of receptor signals by beta-arrestins. *Science* **308**:512–517.
- Liggett SB, Cresci S, Kelly RJ, Syed FM, Matkovich SJ, Hahn HS, Diwan A, Martini JS, Sparks L, Parekh RR, et al. (2008) A GRK5 polymorphism that inhibits beta-adrenergic receptor signaling is protective in heart failure. *Nat Med* **14**:510–517.
- Luciano F, Jacquel A, Colosetti P, Herrant M, Cagnol S, Pages G, and Auberger P (2003) Phosphorylation of Bim-EL by Erk1/2 on serine 69 promotes its degradation via the proteasome pathway and regulates its proapoptotic function. *Oncogene* **22**:6785–6793.
- Luttrell LM, Hawes BE, van Biesen T, Luttrell DK, Lansing TJ, and Lefkowitz RJ (1996) Role of c-Src tyrosine kinase in G protein-coupled receptor- and Gbetagamma subunit-mediated activation of mitogen-activated protein kinases. *J Biol Chem* **271**:19443–19450.
- Mangmool S, Shukla AK, and Rockman HA (2010) beta-Arrestin-dependent activation of Ca(2+)/calmodulin kinase II after beta(1)-adrenergic receptor stimulation. *J Cell Biol* **189**:573–587.
- Martin NP, Whalen EJ, Zamah MA, Pierce KL, and Lefkowitz RJ (2004) PKA-mediated phosphorylation of the beta1-adrenergic receptor promotes Gs/Gi switching. *Cell Signal* **16**:1397–1403.
- Maudsley S, Pierce KL, Zamah AM, Miller WE, Ahn S, Daaka Y, Lefkowitz RJ, and Luttrell LM (2000) The beta(2)-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. *J Biol Chem* **275**:9572–9580.
- McKay MM and Morrison DK (2007) Integrating signals from RTKs to ERK/MAPK. *Oncogene* **26**:3113–3121.
- Mialet Perez J, Rathz DA, Petrashevskaya NN, Hahn HS, Wagoner LE, Schwartz A, Dorn GW, and Liggett SB (2003) Beta 1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. *Nat Med* **9**:1300–1305.
- Noma T, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J, Le Corvoisier P, Violin JD, Wei H, Lefkowitz RJ, et al. (2007) Beta-arrestin-mediated beta1-adrenergic receptor transactivation of the EGFR confers cardioprotection. *J Clin Invest* **117**:2445–2458.
- Packer M, Fowler MB, Roecker EB, Coats AJ, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Staiger C, et al. (2002) Effect of carvedilol on the morbidity of patients with severe chronic heart failure: results of the carvedilol prospective randomized cumulative survival (COPERNICUS) study. *Circulation* **106**:2194–2199.
- Pierce KL, Maudsley S, Daaka Y, Luttrell LM, and Lefkowitz RJ (2000) Role of endocytosis in the activation of the extracellular signal-regulated kinase cascade by sequestering and nonsequestering G protein-coupled receptors. *Proc Natl Acad Sci USA* **97**:1489–1494.
- Rajagopal K, Whalen EJ, Violin JD, Stiber JA, Rosenberg PB, Premont RT, Coffman TM, Rockman HA, and Lefkowitz RJ (2006) Beta-arrestin2-mediated inotropic effects of the angiotensin II type 1A receptor in isolated cardiac myocytes. *Proc Natl Acad Sci USA* **103**:16284–16289.
- Rajagopal S, Rajagopal K, and Lefkowitz RJ (2010) Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat Rev Drug Discov* **9**:373–386.
- Rakesh K, Yoo B, Kim IM, Salazar N, Kim KS, and Rockman HA (2010) beta-Arrestin-biased agonism of the angiotensin receptor induced by mechanical stress. *Sci Signal* **3**:ra46.
- Rochais F, Vilardaga JP, Nikolaev VO, Bünemann M, Lohse MJ, and Engelhardt S (2007) Real-time optical recording of beta1-adrenergic receptor activation reveals supersensitivity of the Arg389 variant to carvedilol. *J Clin Invest* **117**:229–235.
- Rockman HA, Koch WJ, and Lefkowitz RJ (2002) Seven-transmembrane-spanning receptors and heart function. *Nature* **415**:206–212.
- Rozengurt E (2007) Mitogenic signaling pathways induced by G protein-coupled receptors. *J Cell Physiol* **213**:589–602.
- Sehnert AJ, Daniels SE, Elashoff M, Wingrove JA, Burrow CR, Horne B, Muhlestein JB, Donahue M, Liggett SB, Anderson JL, et al. (2008) Lack of association between adrenergic receptor genotypes and survival in heart failure patients treated with carvedilol or metoprolol. *J Am Coll Cardiol* **52**:644–651.
- Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S, Reiter E, Premont RT, Lichtarge O, and Lefkowitz RJ (2006) beta-arrestin-dependent, G protein-independent ERK1/2 activation by the beta2 adrenergic receptor. *J Biol Chem* **281**:1261–1273.
- Shin J, Lohmeyer MT, Gong Y, Zineh I, Langaee TY, Yarandi H, Schofield RS, Aranda JM Jr, Hill JA, Pauly DF, et al. (2007) Relation of beta(2)-adrenoceptor haplotype to risk of death and heart transplantation in patients with heart failure. *Am J Cardiol* **99**:250–255.
- Shukla AK, Violin JD, Whalen EJ, Gesty-Palmer D, Shenoy SK, and Lefkowitz RJ (2008) Distinct conformational changes in beta-arrestin report biased agonism at seven-transmembrane receptors. *Proc Natl Acad Sci USA* **105**:9988–9993.
- Spiering W, Kroon AA, Fuss-Lejeune MM, Daemen MJ, and de Leeuw PW (2000) Angiotensin II sensitivity is associated with the angiotensin II type 1 receptor A(1166)C polymorphism in essential hypertensives on a high sodium diet. *Hypertension* **36**:411–416.
- Stefanovsky V, Langlois F, Gagnon-Kugler T, Rothblum LI, and Moss T (2006) Growth factor signaling regulates elongation of RNA polymerase I transcription in mammals via UBF phosphorylation and r-chromatin remodeling. *Mol Cell* **21**:629–639.
- Strachan RT, Sciaky N, Cronan MR, Kroeze WK, and Roth BL (2010) Genetic deletion of p90 ribosomal S6 kinase 2 alters patterns of 5-hydroxytryptamine 2A serotonin receptor functional selectivity. *Mol Pharmacol* **77**:327–338.
- Tohgo A, Choy EW, Gesty-Palmer D, Pierce KL, Laporte S, Oakley RH, Caron MG, Lefkowitz RJ, and Luttrell LM (2003) The stability of the G protein-coupled receptor-beta-arrestin interaction determines the mechanism and functional consequence of ERK activation. *J Biol Chem* **278**:6258–6267.
- Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch

- JA, Roth BL, Christopoulos A, Sexton PM, et al. (2007) Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther* **320**:1–13.
- Violin JD, Ren XR, and Lefkowitz RJ (2006) G-protein-coupled receptor kinase specificity for beta-arrestin recruitment to the beta2-adrenergic receptor revealed by fluorescence resonance energy transfer. *J Biol Chem* **281**:20577–20588.
- Waskiewicz AJ, Flynn A, Proud CG, and Cooper JA (1997) Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J* **16**:1909–1920.
- Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM, and Lefkowitz RJ (2003) Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc Natl Acad Sci USA* **100**:10782–10787.
- Wisler JW, DeWire SM, Whalen EJ, Violin JD, Drake MT, Ahn S, Shenoy SK, and Lefkowitz RJ (2007) A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proc Natl Acad Sci USA* **104**:16657–16662.
- Xiao K, McClatchy DB, Shukla AK, Zhao Y, Chen M, Shenoy SK, Yates JR 3rd, and Lefkowitz RJ (2007) Functional specialization of beta-arrestin interactions revealed by proteomic analysis. *Proc Natl Acad Sci USA* **104**:12011–12016.
- Xiao K, Sun J, Kim J, Rajagopal S, Zhai B, Villén J, Haas W, Kovacs JJ, Shukla AK,

Hara MR, et al. (2010) Global phosphorylation analysis of beta-arrestin-mediated signaling downstream of a seven transmembrane receptor (7TMR). *Proc Natl Acad Sci USA* **107**:15299–15304.

- Zhai P, Yamamoto M, Galeotti J, Liu J, Masurekar M, Thaisz J, Irie K, Holle E, Yu X, Kupersmidt S, et al. (2005) Cardiac-specific overexpression of AT1 receptor mutant lacking G alpha q/G alpha i coupling causes hypertrophy and bradycardia in transgenic mice. *J Clin Invest* **115**:3045–3056.
- Zhu WZ, Zheng M, Koch WJ, Lefkowitz RJ, Kobilka BK, and Xiao RP (2001) Dual modulation of cell survival and cell death by beta(2)-adrenergic signaling in adult mouse cardiac myocytes. *Proc Natl Acad Sci USA* **98**:1607–1612.
- Zou Y, Komuro I, Yamazaki T, Kudoh S, Aikawa R, Zhu W, Shiojima I, Hiroi Y, Tobe K, Kadowaki T, et al. (1998) Cell type-specific angiotensin II-evoked signal transduction pathways: critical roles of Gbetagamma subunit, Src family, and Ras in cardiac fibroblasts. *Circ Res* **82**:337–345.

Address correspondence to: Dr. Howard A Rockman, Duke University Medical Center 3104, Durham, NC 27710. E-mail: h.rockman@duke.edu