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Review Article

GRK2 and β-arrestins in cardiovascular disease: Something old, something new

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Abstract: Heptahelical G protein-coupled receptors are the most diverse and therapeutically important family of receptors in the human genome, playing major roles in the physiology of various organs/tissues including the heart and blood vessels. Ligand binding activates heterotrimeric G proteins that transmit intracellular signals by regulating effector enzymes or ion channels. G protein signaling is terminated, in large part, by phosphorylation of the agonist-bound receptor by the family of G-protein coupled receptor kinases (GRKs) followed by βarrestin binding, which uncouples the phosphorylated receptor and G protein and subsequently targets the receptor for internalization. As the receptor-βarrestin complex enters the cell, βarrestins serve as ligand-regulated scaffolds that recruit a host of intracellular proteins and signal transducers, thus promoting their own wave of signal transduction independently of G-proteins. A large number of preclinical studies in small and large animals over the past several years have pinpointed specific pathophysiologic roles played by these two families of receptor-regulating proteins in various cardiovascular diseases, directly implicating them in disease pathology and suggesting them as potential therapeutic targets. The present review gives an account of what is currently known about the benefits of cardiac GRK2 inhibition for cardiovascular disease treatment, and also discusses the exciting new therapeutic possibilities emerging from uncovering the physiological roles of adrenal GRK2 and of βarrestin-mediated signaling in vivo in the cardiovascular system.

Keywords: heptahelical receptor, cardiovascular disease, β-adrenergic receptor, GRK2, βarrestin signaling, adrenal gland, catecholamine, angiotensin II type 1 receptor, vascular smooth muscle

Introduction

The heptahelical or seven-transmembrane spanning receptors (7TMRs) or G protein-coupled receptors (GPCRs) are by far the largest and most diverse superfamily of cell surface receptors. Approximately 600 distinct genes encoding non-olfactory GPCRs make up greater than 1% of the human genome [1-2]. Such evolutionary diversity enables GPCRs to detect an extraordinary array of extracellular stimuli. GPCRs function in neurotransmission, neuroendocrine control of physiologic homeostasis and reproduction, and regulation of hemodynamics and intermediary metabolism, and they control the growth, proliferation, differentiation, and death of multiple cell types. It is estimated that more than half of all drugs in clinical use target GPCRs, acting either to mimic endogenous GPCR ligands, to block ligand access to the receptor, or to modulate ligand production [3]. Agonist binding promotes interaction of the receptor with heterotrimeric G proteins, which initiates the classical intracellular signaling of these receptors that ultimately leads to a variety of cellular responses/physiological effects.

At the same time, agonist binding promotes the phenomenon of homologous or agonist-dependent receptor desensitization, which is the molecular basis of the waning of the cellular responsiveness to persistent receptor stimulation and constitutes a major classical homeostatic mechanism of cellular physiology [4]. Agonist-dependent desensitization is conferred, at the molecular level, by phosphorylation of the receptor by the family of kinases known as G-protein-coupled receptor kinases (GRKs). GRKs are serine-threonine protein kinases comprising seven isoforms (GRK1-7). The CT domain of

GRKs contributes to their subcellular localization and agonist-dependent translocation by favoring their interaction with lipids and other membrane proteins. GRK2, GRK3 and GRK5 are ubiquitously expressed, including the heart where GRK2 represents the most abundant isoform [4]. GRK2 is a cytosolic protein in its inactive state, which recognizes, binds and phosphorylates agonist-occupied GPCRs, amongst them β- and α_2-adrenergic receptors (βARs and α_2ARs) and angiotensin II type 1 receptors (AT_1Rs) [5]. When the receptor is activated by agonist, transient conformational changes in the receptor lead to heterotrimeric G -protein activation by dissociation of G_s from $G_{\beta\gamma}$ subunits. The CT of GRK2 (GRK2ct or βARKct) interacts with the free $G_{\beta\gamma}$ subunits resulting in translocation of GRK2 to the plasma membrane where it can phosphorylate intracellular domains of agonist-occupied GPCR [4-7]. The phosphorylation enhances the affinity of the receptor for binding to the adapter proteins βarrestins (βarrs), which sterically prevent further G protein activation and signaling [4]. Furthermore, the binding of βarrs to the receptor promotes internalization of activated receptors to endosomal compartments for subsequent recycling (resensitization) or degradation (downregulation). In addition, the βarr-receptor complex is able to elicit new, G protein-independent, signals as it traffics through the intracellular compartments [8-9].

Here, we will review the current literature regarding the cardiovascular roles of GRK2 and βarrs with a focus on the more established therapeutic value of cardiac GRK2 targeting, as well as on the emerging roles of adrenal GRK2 and cardiovascular (CV) βarr-dependent signaling in CV pathophysiology. For more extensive reviews of the physiological roles of βarr signaling in vitro and in vivo, the reader is referred to several excellent recent reviews [7-10].

Cardiac GRK2 in CV disease

The main target of cardiomyocyte GRK2 is the cardiac βAR. βARs are among the most important GPCRs in CV physiology regulation. Their principal role in the heart is the regulation of cardiac rate and contractility in response to the catecholamines (CAs) norepinephrine (NE) and epinephrine (Epi). βARs comprise three subtypes, β_1, β_2 and β_3, each one with its own functional and molecular properties. β_1AR is the predominant subtype in the myocardium, representing 75-80% of total βAR density, followed by β_2AR, which comprises about 15-18% of total cardiomyocyte βARs and the remaining 2-3% is β_3ARs (under normal conditions) [11]. β_1AR stimulation by catecholamines results in the dissociation of the stimulatory G protein alpha subunit ($G_{\alpha s}$) from $G_{\beta\gamma}$. $G_{\alpha s}$ stimulates adenylyl cyclase (AC) to produce the second messenger cyclic adenosine monophosphate (cAMP), which, by activating protein kinase A (PKA), regulates a plethora of intracellular, sarcolemmal and myofibrillar substrates, thus exerting the cellular effects of receptor activation on cardiac chronotropy, inotropy and lusitropy. In addition, $G_{\beta\gamma}$ can also activate downstream effectors that participate in cardiac signaling and function [11]. β_2AR also mediates the effects of catecholamines on the heart, but in a qualitatively different manner from β_1AR, as it can also couple to the AC inhibitory G protein (G_i). It is now generally accepted that in the heart, β_2AR signals and functions in a substantially different manner compared to β_1AR [12-14]. Importantly, whereas β_1AR activation enhances cardiomyocyte apoptosis, β_2AR exerts antiapoptotic effects in the heart [12-14]. This essential difference between the two receptor subtypes is ascribed to the signal of β_2AR through G_i [12-13]. Studies using transgenic mice, β_2AR-selective stimulation, and adenoviral-mediated β_2AR overexpression, have demonstrated the protective effects of β_2AR signaling in the myocardium, including improved cardiac function and decreased apoptosis. Conversely, hyperstimulation or overexpression of β_1AR has detrimental effects in the heart [15-16].

Of note, the differences between the two predominant cardiac βARs (β_1AR & β_2AR), in terms of their signaling properties, might take a quite different shape and have a much bigger bearing on pathophysiologic implications in the setting of HF: for instance, and as discussed in more detail below, β_1AR is selectively downregulated (i.e. functional receptor number reduced) in heart failure (HF), thus shifting the aforementioned β_1AR:β_2AR ratio towards 50:50 in the failing heart from 75:~25 in the normal, healthy heart [17-18]. However, β_2AR is also nonfunctional and does not signal properly in the failing heart (again see below) [6-17]. In addition, emerging evidence suggests that β_2AR signaling in the failing heart is quite different from that in the normal heart, i.e. is more dif-

fuse and non-compartmentalized and resembles more the pro-apoptotic "diffuse" cAMP signaling pattern of the β_1AR [19]. Therefore, this stoichiometric shift in favor of the supposedly "good" β_2AR in HF appears unable to help the heart improve its structure and function.

During HF, several neurohormonal systems are hyperactive; in particular, elevated sympathetic nervous system (SNS) activity and outflow, characterized by an increase in the circulating levels of Epi and NE, is a hallmark of chronic HF [20-21]. It is widely recognized that chronically elevated stimulation of the β-adrenergic system exerts toxic effects on the heart and plays a key pathogenic role in HF progression. Overall, success of β-blocker treatment at reducing HF progression and related morbidity and mortality, is attributable to the ability of these drugs to protect the heart from the detrimental effects of elevated CAs [22-23]. On the other hand, administration of β-agonists, despite producing immediate hemodynamic benefits, reduces the overall survival of chronic HF patients [24]. It has now been almost 30 years since Bristow and colleagues described the reduced cardiac βAR density and impaired inotropic response to adrenergic stimulation in the human failing heart for the first time. Further investigations over the following decade have clarified the molecular changes involving the cardiac βAR system that take place during HF development, and it is now well known that the chronically elevated CA stimulation causes significant derangements of βAR signaling and function in HF [17-18-21]. βAR dysfunction is characterized at the molecular level by selective reduction of β_1AR density at the plasma membrane (downregulation) and by uncoupling of the remaining membrane β_1ARs and β_2ARs from G proteins (desensitization) [17]. Importantly, during HF, levels and activities of both myocardial GRK2 and GRK5 have been shown to be elevated, both in humans and in animal models of the disease. Increasing interest into the role GRK2 plays in cardiovascular pathophysiology is due to the fact that this kinase is upregulated in several different pathologic conditions, such as cardiac ischemia, hypertrophy, and hypertension [25-31]. In HF, cardiac GRK2 protein levels are elevated in the early stages of the disease and several lines of evidence suggest this kinase can serve as a potential novel biomarker of cardiac dysfunction in human HF [31-33]. Currently, the general consensus is that the excessive amount of CAs "hitting" the receptor from its extracellular side is an early trigger for increased GRK2 levels/activity in HF, thus leading to a reduction in βAR density and responsiveness and resulting in further deterioration of cardiac function [34].

Theoretically, the upregulation of GRK2 observed in HF could be interpreted as a homeostatic protective mechanism aiming to defend the heart from the toxic effects of excessive catecholaminergic stimulation by reducing signaling through βARs. However, about 15 years ago the first report came out demonstrating that GRK2 upregulation is detrimental for the heart and causes the functional uncoupling of βAR *in vivo* [35]. This prompted investigations of the role GRK2 plays in the heart, which uncovered the crucial importance of this kinase in regulating cardiac contractility and function. Specifically, cardiomyocyte-restricted overexpression of GRK2 to the same level of upregulation found in human HF (i.e. 3–4 fold) markedly attenuated *in vivo* βAR signaling and contractile reserve, showing that indeed this GRK could functionally uncouple endogenous receptors [36]. Contractile response to angiotensin II stimulation was also blunted in these mice suggesting that GRK2 is targeting other receptor systems in the heart, as well [37]. In order to inhibit GRK2 activity specifically in the heart *in vivo*, cardiac-specific βARKct transgenic mice were subsequently generated in order to prevent Gβγ-mediated membrane translocation/activation of the kinase [36]. Importantly, in a "proof of concept" type of study for the physiological role of GRK2 in the heart, βARKct expression in the heart enhanced cardiac contractility both at baseline and after adrenergic stimulation [36]. Moreover, cardiac βARKct expression was able to reverse the contractile and βAR dysfunctions due to transgenic overexpression of GRK2 [38]. These findings clearly demonstrated that levels of GRK2 activity in the heart are a major determinant of cardiac performance with enhanced GRK2 activity being a negative regulator of cardiac contractile function and thus, lowering GRK2 activity has positive inotropic properties in the heart (**Figure 1**). Following these initial studies, the hypothesis that myocardial GRK2 elevation during disease progression is maladaptive was subsequently tested and confirmed in a vast variety of genetic mouse models of HF, such as the muscle LIM-domain protein (MLP) knockout (KO) mice [39], mice with

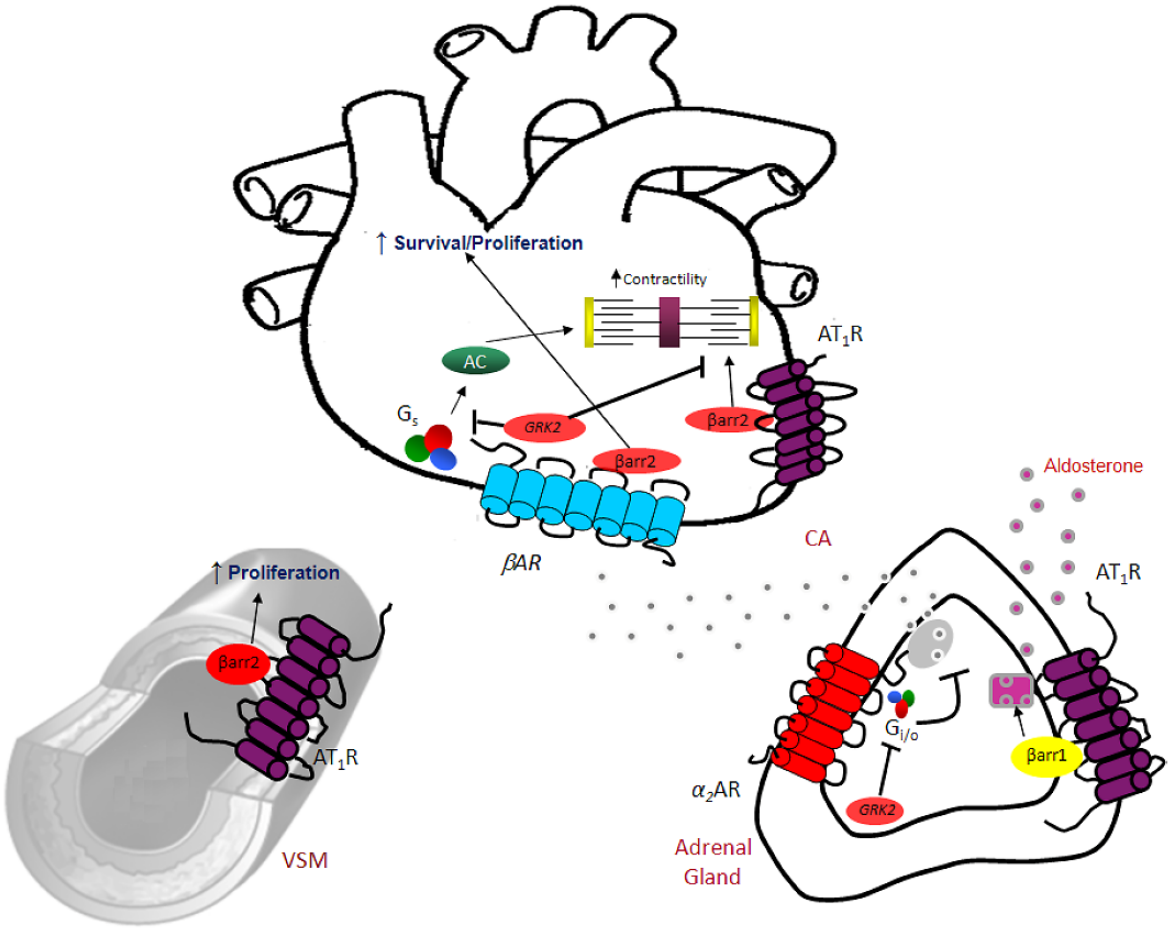

Figure 1. Cardiovascular roles of GRK2 and βarrs with potential for therapeutic exploitation in cardiovascular disease (see text for details). CA: catecholamine (epinephrine or norepinephrine); G_s: stimulatory G-protein; $G_{i/o}$: inhibitory or other G-protein; AC: adenylyl cyclase; βAR: beta-adrenergic receptor (β_1 or β_2); GRK2: G-protein coupled receptor kinase-2 (βARK1); α_2AR: alpha2-adrenergic receptor; AT_1R: angiotensin II type 1 receptor; βarr1: beta-arrestin1; β arr2: beta-arrestin2; VSM: vascular smooth muscle.

cardiac-specific overexpression of the Ca^{2+}-binding protein calsequestrin (CSQ) [40], in which βARKct not only improved cardiac function but also prolonged survival in a synergistic manner with β-blockers [40], and in a mouse model of hypertrophic cardiomyopathy [41]. Since βARKct works by sequestering free $G_{\beta\gamma}$ subunits of activated heterotrimeric G-proteins, its beneficial effects might be at least partly mediated by prevention of $G_{\beta\gamma}$-mediated signaling. Thus, the definite proof for GRK2 being pathological in HF progression had to come from mouse KO models. These studies however initially proved very tough to perform since GRK2 KO mice were surprisingly found to be embryonic-lethal [42]. This problem was subsequently tackled by the creation of heterozygous GRK2 KO mice, which develop normally and have 50% of the normal expression levels of GRK2 in the heart. Cardiac function and βAR-mediated inotropic reserve in these mice were found to be similar to βARKct mice [43]. More recently, by taking advantage of the availability of conditional (i.e. tissue- and/or stimulus-specific), cardiac-specific GRK2 KO mice, the critical role of GRK2 in HF pathogenesis and progression has been confirmed [44]. Specifically, mice having cardiomyocyte GRK2 knocked down from birth underwent myocardial infarction (MI) and HF progression was prevented, as well as the characteristic βAR signaling abnormalities present in control post-MI mice [45]. In the very same study, GRK2 gene ablation was also done in myocytes 10 days after MI and this

again prevented subsequent death, reversed pathological LV remodeling, and actually increased cardiac function [45]. Taken together, the results from all these studies in cardiac βARKct-expressing and cardiac-specific GRK2 KO mice, provide compelling evidence for the notion that enhanced GRK2 expression observed in HF is detrimental for the heart and strongly support the concept of cardiac GRK2 inhibition using the βARKct as a promising gene therapy strategy to increase contractility and function of the failing heart (**Figure 1**).

Adrenal GRK2 in CV disease

As mentioned above, one of the most common and devastating pathophysiological features of HF are SNS hyperactivity and outflow, reflected by increased levels of circulating CAs, Epi and NE [20-22-46-47]. Initially an adaptive mechanism aiming to compensate decreased contractility following cardiac insult, it becomes progressively maladaptive, contributing to HF establishment and progression and to its morbidity and mortality [21-22]. At any given time, the circulating CAs that stimulate the heart come from two main sources in the body: sympathetic nerve terminals, releasing NE, and the adrenal medulla, secreting mainly Epi (and also some NE) [48]. This latter process is dependent on tonic activation of nicotinic cholinergic receptors by acetylcholine, which cause release of CAs from the chromaffin cells of the adrenal medulla, and is fine-tuned by α_2ARs, acting as presynaptic inhibitory autoreceptors [49-51]. α_2ARs, similarly to cardiac βARs, are also substrates for agonist-dependent desensitization via GRKs and subsequent downregulation [52-56]. Of note, increased GRK2 expression and activity are observed in the adrenal medulla during HF, which critically influence CA secretion from this source [57]. In particular, our studies over the past few years have documented that adrenal GRK2 overexpression is responsible for a severe adrenal α_2AR dysfunction in chronic HF, which causes a loss of the sympatholytic (i.e. CA-reducing) function of this receptor type in the adrenal gland, thus CA secretion is chronically elevated [57-60]. This emerging crucial role for adrenal GRK2 in HF is underlined by the fact that its inhibition, via adenoviral-mediated βARKct adrenal-specific gene delivery, leads to a significant reduction in CA circulating levels, "resetting" not only adrenal, but also cardiac function [57]. In fact, post-MI HF rats treated with intra-adrenal βARKct gene injection show improved myocardial contractility and cardiac βAR signaling [57]. Therefore, it appears that there is a significant crosstalk at the level of entire organs in a complex syndrome such as HF. Furthermore, since adrenal GRK2 emerges as a crucial regulator of circulating CA levels in HF, targeting it to restore α_2AR function in the adrenal medulla and reduce CA secretion from this source can be envisioned as an attractive sympatholytic strategy for HF treatment [57-60] (**Figure 1**).

Additional evidence for the benefits of adrenal GRK2 inhibition in HF comes from phenylethanolamine-N-methyl transferase (PNMT)-driven GRK2 KO mice. By taking advantage of the Cre/*lox*P technology, we were able to genetically delete GRK2 only in Epi-producing cells (i.e. mainly adrenal chromaffin cells, but also some other sympathetic nervous cell populations capable of converting NE to Epi and secreting the latter) of transgenic mice [58]. This was achieved by means of excision of the "floxed" GRK2 gene specifically in cells that express the PNMT enzyme. When these mice, which do not express GRK2 in their adrenal medullae from birth, underwent surgical MI and were left to develop and establish HF, circulating CAs were significantly reduced over the course of their disease and their cardiac function and dimensions, as well as their cardiac βAR reserve, were all substantially improved [58]. Thus, this study strongly indicates that preventing the catecholaminergic "rush" that hits the heart immediately after an MI, by inhibiting adrenal GRK2 before HF sets in, can help the heart work close to normally and avoid major tissue damage, which is otherwise inevitable in the first few weeks following a heart attack. Thus, this could be another benefit from using adrenal GRK2 inhibition as a sympatholytic strategy in HF as early as possible during the course of the disease (in a similar, and perhaps even complementary, manner to the usage of β-blockers early after an MI in humans).

Adrenal GRK2 has also been shown to critically modulate CA secretion under normal conditions. In fact, in normal healthy rats, adrenal βARKct gene transfer resulted in reduced plasma circulating CA levels; in contrast, GRK2 overexpression in the adrenal medulla led to increased Epi and NE plasma levels [59]. Adding to the importance of adrenal GRK2 in HF, it was recently

shown that exercise training, which exerts several beneficial effects on the cardiovascular system including reduction of HF-related SNS hyperactivity, is also able to normalize GRK2 expression and restore α_2AR function in the adrenal glands of HF rats [60]. It is quite plausible that, during HF, GRK2-dependent α_2AR dysfunction also occurs in the peripheral sympathetic nerve terminals in the heart and other organs, thus contributing to the increased NE release also from these sources. Therefore, systemic GRK2 inhibition might globally lower CA levels, which argues in favor of a small molecule GRK2 inhibitor.

The results of all the above studies propose that GRK2 inhibition be a novel sympatholytic strategy in HF, blocking CA release at the sources of these hormones and preventing their toxic effects on peripheral organs, like the heart. In addition, adrenal βARKct expression might have a synergistic action with β-blockers, as both of these therapeutic strategies target adrenergic hyperactivity in HF. However, while β-blockers ameliorate adrenergic inotropic response in the failing heart by directly protecting the heart from CA hyperstimulation and the increased myocardial GRK2 levels, adrenal GRK2 inhibition could also counteract the extracardiac effects of CAs, including the activation of the endothelin and the renin–angiotensin–aldosterone systems. Additionally, β-blockers directly antagonize the cardio-stimulatory actions of CAs and thus are contraindicated in the acute setting of HF. Adrenal GRK2 inhibition, by lowering global circulating CA levels independently of the heart, might thus be a safer, and much more compatible with drug therapy, sympatholytic approach than β-blockers in acute episodes of HF. Moreover, adrenal GRK2 inhibition might allow for reduction of dosage, and hence of the adverse effects of β-blockers in HF therapy.

Cardiac βarrs and CV disease

βarrs comprise two ubiquitously expressed isoforms, βarr1 and βarr2 (arrestin-2 and -3 respectively), both of which are abundantly expressed in cardiac muscle. As cofactors of GRKs in βAR desensitization/downregulation, they contribute to the diminished inotropic and adrenergic reserve of the failing heart and their inhibition should theoretically be beneficial in acute HF, as it would enhance the $G_{\alpha s}$-AC-PKA axis of pro-contractile signaling of cardiac βARs (see above, under "Cardiac GRK2 in CV disease") thereby increasing cardiac contractility. However, and exactly because βarrs do a lot more than merely ceasing ("arresting") G protein-mediated signaling (i.e. they actually promote signaling in their own right), a number of recent studies point to a beneficial role played by βarrs in the heart, especially when they engage the cardiac β_1AR. In HF, chronic catecholamine stimulation of the β_1AR promotes cardiac hypertrophy, decreased contractility, and increased myocyte apoptosis [35-61]. As a result, administration of β-blockers is currently part of standard care in the clinical management of congestive heart failure. In transfected HEK293 cells, βarrs were found to mediate the mitogenic signaling of EGF (Epidermal Growth Factor) receptor transactivation by the β_1AR. Consistent with this, a mutant β_1AR lacking 14 GRK phosphorylation sites in its CT tail that cannot undergo βarr-dependent desensitization, fails to transactivate the EGF receptors. In response to chronic isoproterenol stimulation, transgenic mice expressing this β_1AR mutant develop severe dilated cardiomyopathy with significantly increased left ventricular (LV) dilatation, decreased fractional shortening, and increased myocardial apoptosis compared with wild-type β_1AR-expressing transgenic mice. In this model, inhibition of EGF receptors worsens the dilated cardiomyopathy, suggesting a protective rather than deleterious role for transactivated EGF receptors in the heart [62] and prompting the investigators to speculate that βarr-dependent EGF receptor transactivation exerts a cardioprotective effect and thus, βarr-mediated (in contrast to the classical G protein-dependent) β_1AR signaling might be of therapeutic benefit in HF (**Figure 1**).

Effects of cardiac βarr-dependent signaling can be quite different when a different receptor is bound by the βarr molecule, i.e. the angiotensin II (AngII) receptor AT_1R, another very important heptahelical receptor in CV physiology and pathology. An artificially constructed AT_{1A}R mutant (AT1-i2m), which fails to activate G proteins but nonetheless interacts with βarrs, activates the mitogenic Src-Ras-ERK1/2 pathway in vitro [63]. In vivo, cardiomyocyte-specific overexpression of this receptor mutant leads to greater cardiomyocyte hypertrophy, bradycardia, and fetal cardiac gene expression than comparable overexpression of the wild type receptor. Conversely, overexpressed wild-type AT_{1A}R produces

greater cardiomyocyte apoptosis and interstitial fibrosis than the G protein-uncoupled mutant, suggesting that G protein-dependent and –independent $AT_{1A}R$ signals mediate different aspects of the hypertrophic response [64]. Of course, these studies do not directly implicate βarr signaling; another series of studies using the AngII peptide analog SII ([Sar^1-Ile^4-Ile^8]-AngII), which, when bound to the $AT_{1A}R$, elicits βarr signaling but not G protein signaling [65], provide direct evidence for potential roles of βarrs in cardiac $AT_{1A}R$ signaling. In primary cardiomyocytes, SII stimulates cardiomyocyte proliferation independently of G proteins, but not hypertrophy, which requires $G_{q/11}$ protein signaling [66]. In addition, SII produces positive inotropic and lusitropic effects on isolated murine cardiomyocytes [67]. These effects require GRK6 and βarr2, whereas GRK2 seems to oppose them, consistent with the specialized role of GRK isoforms described in a transfected system [68]. On the other hand, SII does not produce inotropic or chronotropic effects in isolated Langendorff-perfused cardiac preparations despite its ability to activate ERK (Extracellular signal-Regulated Kinase) 1/2 [66]. Thus, it seems that the $AT_{1A}R$ promotes cardiac hypertrophy and cardiomyocyte proliferation via its classical $G_{q/11}$ protein-PKC (Protein Kinase C) signaling on one hand, and on the other hand increases cardiac contractility via βarr2 signaling (**Figure 1**). Since βarr2 is bound to stop the G protein-mediated signaling of the receptor and GRK2 also seems to oppose this pro-contractile signaling of βarr2, stimulation of βarr2 activity and/or GRK2 inhibition at the cardiac $AT_{1A}R$ would appear therapeutically desirable for the treatment of HF and cardiac hypertrophy.

Extracardiac (adrenal & vascular) βarrs and CV disease

Aldosterone is another one of a number of hormones whose levels are elevated in HF and produces a multitude of negative effects on the failing heart, including promotion of post-MI adverse cardiac remodeling and HF progression [69-71]. It is produced and secreted by the adrenocortical zona glomerulosa cells in response to AT_1R activation by AngII [72]. Until recently, the general consensus for AT_1R signaling to aldosterone production was that it proceeded via activation of $G_{q/11}$-proteins, to which the AT_1R normally couples [73]. Recently however, we described a crucial role for βarr1 in mediating this signaling from AT_1R to aldosterone synthesis and secretion in vitro and in vivo [74]. Specifically, βarr1 was found to stimulate sustained ERK1/2 activation and subsequent upregulation of steroidogenic acute regulatory protein (StAR), a steroid transport protein that catalyzes the rate-limting step in adrenal steroid biosynthesis. Moreover, βarr1 was shown to promote aldosterone production independently of G proteins, since SII could recapitulate the effects of AngII on aldosterone turnover [74]. Importantly in vivo, adrenal βarr1 was shown to be a major regulator of circulating aldosterone levels under normal healthy conditions, since its upregulation, specifically in the adrenal gland, caused hyperaldosteronism in normal healthy animals [74]. Subsequently, we investigated the effects of adrenal βarr1 on aldosterone levels also in diseased animals, as they progressed to HF after a vast experimental MI [75]. We found that adrenal βarr1 overexpression promoted post-MI aldosterone elevation, resulting in accelerated cardiac adverse remodeling and deterioration of LV function. Importantly, these detrimental effects of aldosterone were prevented when adrenal βarr1 was inhibited in vivo via gene therapy with a mini-gene encoding the βarr1 CT domain (βarr1ct), which we designed and developed. Adrenal βarr1 inhibition with this mini-gene markedly reduced circulating aldosterone levels and improved cardiac structure and function in post-MI animals, largely preventing (and/or even reversing) the ensuing cardiac adverse remodeling [75]. Thus, it appears that in the adrenal gland, βarr1 is a major driving force behind elevation of the cardiotoxic aldosterone in HF, and its inhibition might be of therapeutic value in treatment of hyperaldosteronism in HF or post-MI (**Figure 1**). On the other hand, adrenal βarr1, in conjunction with adrenal GRK2, also contributes to adrenal α_2AR desensitization and downregulation which results in chronically elevated CA secretion in HF [57] (see above, under "Adrenal GRK2 in CV disease"). Therefore, adrenal βarr1 inhibition poses as an attractive therapeutic strategy for lowering the neurohormonal burden of the failing heart, since it could represent the proverbial "killing two birds with one stone", i.e. it would lead to lower CAs (sympatholysis) and lower aldosterone, which all contribute significantly to the morbidity and mortality of HF (**Figure 1**).

In vascular smooth muscle (VSM), βarr-dependent signaling appears to promote the development and progression of atherosclerotic vascular disease. Neointimal hyperplasia after carotid endothelial injury is enhanced in βarr1-KO mice but diminished in βarr2-KO mice. Loss of βarr2 appears to decrease GPCR-stimulated VSM cell proliferation and ERK1/2 activation, consistent with a role for βarr2 signaling in the injury response. When the low-density lipoprotein receptor-KO mouse, a genetic model of enhanced atherogenesis, is crossed onto a βarr2-KO background, atheromas and the involved area are significantly reduced [76]. In vitro, both G protein- and βarr-dependent pathways elicited by the $AT_{1A}R$ converge on the EGF receptor in primary VSM cells to stimulate proliferation. Specifically, βarr2 seems to enhance Src-dependent EGF receptor transactivation by the $AT_{1A}R$ [77]. Moreover, βarr2-dependent ERK1/2 activation downregulates the pro-apoptotic phospho-BAD protein, thus inducing anti-apoptotic cytoprotective effects in rat VSM [78]. Thus, it appears that βarr2 can promote $AT_{1A}R$-dependent VSM proliferation and hypertrophy, thereby contributing to the development and progression of atheromas, whereas βarr1 again (similarly to the case of cardiac $AT_{1A}R$ and its pro-contractile signaling) opposes these actions of βarr2. Therefore, in VSM and in atherosclerotic disease, βarr2 inhibition and/or βarr1 stimulation might be of therapeutic value (**Figure 1**).

Another vascular-related physiological effect of βarr-dependent signaling appears to be facilitation of niacin (nicotinic acid)-induced flushing, a major adverse effect of pharmacotherapy with this very useful lipid-lowering drug which frequently limits its use in patients. Through its actions on GPR109A, a heptahelical receptor, nicotinic acid decreases serum free fatty acids but also causes cutaneous blood flow/flushing in humans and in mice [79-80]. It was recently demonstrated that βarrs, although dispensable for the beneficial effects of niacin on free fatty acid levels, actually promote the niacin-related flushing, as measured by perfusion of the ventral ear [81]. This effect was specifically attributed to βarr1 only, since βarr2-KO mice did exhibit the flushing normally observed in wild type mice upon treatment with niacin. The mechanism of this βarr1-dependent effect is niacin-induced βarr1-mediated activation of phospholipase A2, which stimulates arachidonic acid release, the precursor of vasodilatory prostaglandin D2 (PGD2) synthesis [81]. PGD2 is ultimately responsible for the niacin-induced flushing response. Thus, βarr1 inhibition at the GPR109A receptor or a "biased" GPR109A ligand capable of activating G protein signaling without βarr activation would be therapeutically desirable, as it would confer the same therapeutic effect of niacin on lipid metabolism without the adverse effect of flushing. Indeed, a GPR109A partial agonist was developed recently (MK-0354) that decreases serum free fatty acids without producing cutaneous flushing and thus appears superior to niacin for lipid-lowering therapy [82].

Concluding Remarks/Future Directions

An enormous and ever-expanding body of preclinical evidence has been built that strongly supports cardiac GRK2 inhibition as a therapeutic modality for HF. However, recent studies by us and others have begun to establish GRK2 targeting in extracardiac tissues, and more specifically in the adrenal gland and in sympathetic neurons, as another, novel HF therapeutic possibility. Adrenal GRK2 inhibition emerges as an attractive therapeutic strategy to directly lower the neurohormonal (i.e. catecholamines & aldosterone) burden of the failing post-MI heart, without having to intervene in the heart per se.

In addition, the recently emerging and constantly expanding field of heptahelical receptor βarr-dependent signaling also offers several exciting new opprtunities for therapeutic intervention in cardiovascular disease. Despite being still in its infancy, as far as identification of specific physiological and pathpophysiological effects is concerned, there is a huge potential for exploiting this area for therapeutic purposes. Admittedly, the picture is still hazy regarding physiological actions of βarrs, in particular with relation to the cardiovascular system. For instance, it appears that the two βarr isoforms are far from interchangeable when it comes to their physiological actions; quite on the contrary, they actually oppose one another in their ultimate cellular effects most of the time. Therefore, isoform-specific targeting might ultimately be warranted, if targeting of βarr-dependent signaling is ever going to be seriously considered as a therapeutic strategy for the

treatment of cardiovascular disease. Future studies will undoubtedly help clear the picture. In any case, targeting of GRK2 and of βarr-dependent signal transduction represent two very exciting and potentially very promising possibilities for novel therapeutic approaches in cardiovascular disease, a spectrum of disorders in desperate need of new and innovative effective drugs and therapies.

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