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Review

# Targeting GPCR-Gβγ-GRK2 signaling as a novel strategy for treating cardiorenal pathologies

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ABSTRACT

The pathologic crosstalk between the heart and kidney is known as cardiorenal syndrome (CRS). While the specific mechanisms underlying this crosstalk remain poorly understood, CRS is associated with exacerbated dysfunction of either or both organs and reduced survival. Maladaptive fibrotic remodeling is a key component of both heart and kidney failure pathogenesis and progression.

G-protein coupled receptor (GPCR) signaling is a crucial regulator of cardiovascular and renal function. Chronic/pathologic GPCR signaling elicits the interaction of the G-protein Gβγ subunit with GPCR kinase 2 (GRK2), targeting the receptor for internalization, scaffolding to pathologic signals, and receptor degradation. Targeting this pathologic Gβγ-GRK2 interaction has been suggested as a possible strategy for the treatment of HF. In the current review, we discuss recent updates in understanding the role of GPCR-Gβγ-GRK2 signaling as a crucial mediator of maladaptive organ remodeling detected in HF and kidney dysfunction, with specific attention to small molecule-mediated inhibition of pathologic Gβγ-GRK2 interactions. Further, we explore the potential of GPCR-Gβγ-GRK2 signaling as a possible therapeutic target for cardiorenal pathologies.



## 1. Introduction

Cardiovascular diseases (CVD) involve heart and blood vessels and include coronary artery disease (CAD), stroke, hypertension, congenital heart disease, cardiomyopathy, etc. [1]. CVD is the leading cause of death worldwide that accounts for more than 17.3 million deaths per year [2]. In 2013, CVD represented about one of every three deaths in America. Over 85 million Americans are living with some type of CVD or the after-effects of stroke. Projected costs of CVD including the cost of health care services, medications and loss of productivity totals more than $600 billion in 2015, and it is expected to grow to more than $1200 billion by 2030 [3].

Heart failure (HF) is a chronic, progressive condition in which the heart muscle is unable to pump enough blood to meet the body's metabolic demands. HF arises as the final manifestation of many CVDs such as coronary artery disease, congenital malformations and hypertension. About 5.7 million adults in the United States are affected by this debilitating disease [3]; HF treatment costs the nation an estimated $30.7 billion each year [4]. Notwithstanding significant advances in HF treatment and management realized with β-adrenergic receptor (β-AR) blockers, angiotensin receptor blockers, angiotensin converting enzyme (ACE) inhibitors, aldosterone inhibitors, and diuretics, conventional pharmacological therapies only impede the progression and death due to HF, but do not cure it causatively [5]. Taking into consideration the steady growth of aging and diabetic populations, deeper understanding of the molecular and cellular processes that contribute to the disease pathogenesis, along with development of innovative therapeutic strategies allowing the causative cure of HF, are indispensable.

Multiple pathophysiological mechanisms contribute to HF development and progression, including neurohumoral activation [6], G-protein-coupled receptor desensitization and down-regulation [7–9], and extracellular matrix (ECM) mediated pathologic remodeling [10]. Moreover, cardiac pathologies in HF are frequently accompanied by worsening renal function, which is known to be a strong predictor of increased mortality in HF patients [11,12]; this is defined as Cardiorenal syndrome (CRS) type II. In the present review, we discuss recent advances in exploring GPCR signaling as a possible therapeutic target in cardiac disease and as a potential link between failing heart and kidney, with the particular emphasis on small molecule targeting of G-protein βγ subunit - GPCR kinase 2 (Gβγ-GRK2) components of GPCR signaling.

## 2. G-protein-coupled receptor signaling

G-protein-coupled receptors (GPCRs), also known as seven-transmembrane domain receptors, represent a conserved family of receptors that sense molecules outside the cell that activate intracellular signal transduction pathways and consecutive cellular responses. GPCRs are

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integral proteins that comprised an extracellular N-terminus, seven transmembrane (7-TM) α-helixes (TM-1 to TM-7) connected by three intracellular (IL-1 to IL-3) and three extracellular loops (EL-1 to EL-3), and an intracellular C-terminus [13]. Ligand binding to an extracellular active site of the receptor induces a conformational change in the GPCR which allows for coupling with heterotrimeric guanine-nucleotide regulatory proteins (G-proteins) [14]. G-proteins are heterotrimers of α, β and γ subunits known as Gα, Gβ and Gγ, respectively. The heterotrimeric G-proteins are rendered inactive when reversibly bound to Guanosine diphosphate (GDP) but active when bound to Guanosine triphosphate (GTP) [15]. Receptor activation facilitates the exchange of GDP for GTP on Gα subunits that result in dissociation of the Gα from Gβγ subunits to mediate downstream signaling pathways [16]. Dissociated Gα subunits signal via activation of an effector molecule, such as adenyl cyclase (AC) or phospholipase C β (PLCβ) to produce second messengers such as cyclic adenosine 3′, 5′ monophosphate (cAMP), diacylglycerol (DAG), or inositol 1, 4, 5-triphosphate (IP3), respectively. These second messengers modulate a variety of downstream processes, particularly regulation of contractility, hypertrophy, and apoptosis in the heart [15]. Gα proteins are classified into the families $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{11/12}$ [15] with respect to downstream signaling molecules and modulated physiological processes. Dissociated Gβγ subunits target a wide range of signaling pathways involved in receptor desensitization and down-regulation, ion channel activation, enzyme activity modulation, cell division, transcription and cellular organelle function [17–20].

GPCRs respond to extracellular signaling mediated by an extensive amount of agonists such as hormones, proteins and lipids, and participate in a comprehensive variety of physiological processes [21]. In particular, GPCRs play an important role in local and systemic regulation of cardiac function. Specifically, cardiac β-adrenergic receptors (β-ARs) are prominent regulators of cardiovascular chronotropy and inotropy [22,23]. Furthermore, GPCRs mediate a variety of functions in the kidney, and inappropriate activation and regulation of GPCRs may lead to kidney disease [24]. In this review, we focus on Gβγ-mediated signaling as a crucial component of HF pathogenesis and as a potential therapeutic target in cardiorenal pathologies.

## 3. β-Adrenergic receptor signaling in healthy and diseased heart

As mentioned above, cardiac β-ARs represent crucial regulators of cardiac contractile function. In response to sympathetic nervous system (SNS) activity released via mediators, catecholamines (CA) epinephrine (Epi, also named adrenaline) and norepinephrine (NEpi, also named noradrenaline), β-ARs modulate the rate and force of myocardial contractions [8]. There are three β-AR subtypes identified in mammalian hearts: β1, β2, and β3-ARs [25]. Both β1- and β2-ARs are coupled to the downstream excitatory $G\alpha_s$ protein, which generally results in the activation of adenylyl cyclase (AC) and the generation of cyclic AMP (cAMP), eliciting positive chronotropic and inotropic responses. Upon chronic stimulation, β2-ARs also couple to the inhibitory $G\alpha_i$ protein, which has been reported to exert a cardioprotective effect during cardiac injury [26].

In healthy human myocardium, the predominant β-ARs subtypes are the β1- and β2-ARs, which are present in an approximate 80:20 ratio, respectively with only a relatively minor contribution of β3-ARs [27]. Under physiological conditions, β-ARs account for regulation of both heart rate and contractility [14,28]. In HF pathogenesis, excess SNS activation and subsequent catecholamine overdrive is initiated as an adaptation to compensate for decreased heart rate and cardiac contractility and to maintain mean arterial pressure (MAP) [29]. Initially, the elevated SNS activity increases heart rate and contractility through β-AR stimulation. However, maladaptive effects of the elevated SNS activity including myocardial ischemia, pathologic hypertrophy, arrhythmogenicity, myocardial necrosis and apoptosis contribute substantially to disease progression [22,30–32]. This maladaptive response results partially from down-regulation and desensitization of cardiac β-ARs due to chronic CA stimulation [15]. In failing hearts, heightened CA β-AR stimulation induces selective down-regulation of β1-ARs and consequent alteration of the β1-AR to β2-AR ratio from an 80:20 distribution to a ratio of 60:40 [27,33]; the remaining β1-ARs and β2-ARs in failing hearts prevail in a desensitized condition [30].

Cardiac β-AR signaling regulation involves activation-dependent and -independent mechanisms of desensitization [8]. Homologous, agonist-mediated, activation-dependent desensitization is accomplished by an active form of a G-protein-coupled receptor kinase (GRK) that is translocated to the adrenergic receptor after binding with the activated membrane-associated Gβγ subunit to phosphorylate the agonist-occupied receptor [34]. An alternative, activation-independent pathway, known as heterologous desensitization, is accomplished through the activity of a downstream signaling product of β-AR activation or other GPCR signaling events. In both cases, phosphorylated β-AR is bound by β-arrestin molecules which block the access of heterotrimeric G proteins to the receptor thereby uncoupling it and attenuating β-AR signaling in the heart [35,36].

## 4. Gβγ-GRK2 signaling manipulation as a strategy to treat cardiac disease

### 4.1. GRK2: structure, subcellular localization and function in the heart

GRK2 (aka β-adrenergic receptor kinase, βARK) belongs to a family of serine/threonine kinases that share common structural and functional features. Seven mammalian GRKs that have been characterized so far are classified into three subfamilies according to their sequence and structural similarity: (1) the rhodopsin kinase subfamily (GRK1 and GRK7); (2) the βARK subfamily (GRK2 and GRK3); and (3) the GRK4-like subfamily (GRK4, GRK5, GRK6) [37]. Within the cardiovascular system, GRKs 2, 3 and 5 are known to be expressed and play a role in GPCR phosphorylation [38], with GRK2 as a predominant GRK isoform in the heart [39].

GRKs are characterized by a tri-domain structure, with the conserved central catalytic domain and two flanking domains variable in structure in different GRK subfamilies [40]. GRK2's amino (N)-terminal domain that is responsible for receptor recognition and activity regulation contains a regulator of G protein signaling (RGS) homology (RH) domain that has been demonstrated to interact with $G\alpha_q$ proteins [41]. The carboxyl (C)-terminal domain of GRK2 determines membrane targeting and subcellular localization of the enzyme. This domain contains a pleckstrin homology (PH) domain that binds Gβγ subunits [42]. Under basal conditions, GRK2 is distributed primarily in the cytoplasm. Upon GPCR activation, GRK2 is translocated to the plasma membrane via binding with the activated Gβγ subunits. GRK2-mediated phosphorylation of the GPCR causes β-arrestin recruitment to the receptor and consequent inhibition of dissociated G-proteins from coupling to the receptor/β-arrestin complex and further attenuation of downstream signaling [43]. Moreover, β-arrestin-bound receptors are targeted for clathrin-coated pits in the cell membrane that are internalized and either degraded in intracellular lysosomes or recycled back to the cell surface [44].

Apart from the classical mechanism of modulating GPCR signaling in the heart and extracardiac tissues, GRK2 may have other functions independent of GPCR phosphorylation. Recently emerging data suggest the concept of an extensive "GRK2 interactome" that refers to GRK2 interactions with other intracellular proteins such as α-actinin, clathrin, calmodulin, caveolin, tubulin, Akt, HDAC6 and ERK1/2 [39,45]. Investigation of GRK2 functions beyond GPCR desensitization and down-regulation may provide new insights in understanding its role in disease pathogenesis. In the current review, we highlight recent updates relevant for GPCR-Gβγ signaling in HF modulation.

Understanding of the *in vivo* function of GRK2, particularly its role in cardiovascular system function and development, emerged from gene

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