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Invited review

The expanding GRK interactome: Implications in cardiovascular disease and potential for therapeutic development

Jonathan Hullmann[b,1], Christopher J. Traynham[a,1], Ryan C. Coleman[a], Walter J. Koch[a,*]

[a] Center for Translational Medicine, Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140, United States
[b] Thomas Jefferson University, Philadelphia, PA 19107, United States

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ABSTRACT

Heart failure (HF) is a global epidemic with the highest degree of mortality and morbidity of any disease presently studied. G protein-coupled receptors (GPCRs) are prominent regulators of cardiovascular function. Activated GPCRs are "turned off" by GPCR kinases (GRKs) in a process known as "desensitization". GRKs 2 and 5 are highly expressed in the heart, and known to be upregulated in HF.

Over the last 20 years, both GRK2 and GRK5 have been demonstrated to be critical mediators of the molecular alterations that occur in the failing heart. In the present review, we will highlight recent findings that further characterize "non-canonical" GRK signaling observed in HF. Further, we will also present potential therapeutic strategies (i.e. small molecule inhibition, microRNAs, gene therapy) that may have potential in combating the deleterious effects of GRKs in HF.

Contents

Abbreviations: 3′ UTR, 3′ untranslated region; AAV6, adeno-associated virus serotype 6; Ad, adenoviral; AT1R, angiotension receptor; AR, adrenergic receptor; AngII, angiotensin II; C, carboxyl; Ca^{2+}, calcium; CaM, calmodulin; CSQ, calseqestrin; DCM, dilated cardiomyopathy; EGFR, epidermal growth factor; eNOS, endothelial nitric oxide synthase; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; GRK2-C340S, mice wherein GRK2 cannot be *S*-nitrosylated; GRK5-NLS, mice wherein GRK5 cannot be targeted to the nucleus; HDAC, histone deacetylase; Hsp90, heat shock protein 90; HF, heart failure; I/R, ischemia-reperfusion; KO, knockout; LV, left ventricular; MEF2, myocyte enhancer factor 2; MI, myocardial infarction; MLP, muscle lim protein; NLS, nuclear localization sequence; NO, nitric oxide; NOX-4, NADPH oxidase-4; RGS, regulator of G protein signaling; RH, region of homology; ROS, reactive oxygen species; SNS, sympathetic nervous system; SSRI, serotonin reuptake inhibitor; TAC, transverse aortic constriction; TgGRK5, transgenic GRK5.

* Corresponding author at: Department of Pharmacology, Center for Translational Medicine, Temple School of Medicine, MERB 941, 3500 N. Broad Street, Philadelphia, PA 19140, United States.
E-mail address: Walter.Koch@temple.edu (W.J. Koch).
[1] These authors contributed equally to the manuscript.

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1. Introduction

1.1. GPCR signaling

Cellular receptors allow for the transduction and amplification of external environmental stimuli into intracellular signals which initiate various cellular functions. Many classes of receptors exist, with the G protein-coupled receptors (GPCRs) or seven-transmembrane receptors comprising the largest group with over 800 members [1]. GPCRs comprise the largest protein superfamily in the mammalian genome and are able to respond to a wide variety of extracellular signals including photons, small molecules, peptides and proteins [2]. This family of receptors is a major target of therapeutic intervention as most physiological processes require signaling through GPCRs including vision, smell, taste as well as neurologic, cardiovascular, endocrine and reproductive function [3].

Upon ligand binding, GPCRs signal intracellularly via downstream activation of heterotrimeric G-proteins. Activated GPCRs are effectively "turned off" in a process known as "receptor desensitization", whereby the GPCR is phosphorylated by GPCR kinases (GRKs) leading to β-arrestin binding (Fig. 1). GPCR phosphorylation by GRKs increases the affinity of the interaction between the GPCR and β-arrestins ultimately leading to either receptor recycling, degradation or β-arrestin mediated signaling [4,5].

1.2. The GRK family

The seven GRKs are further classified into 3 subfamilies based on sequence and structural similarity: the rhodopsin kinase subfamily (GRK1 and GRK7), the βARK subfamily (GRK2 and GRK3) and the GRK4-like subfamily (GRK4, GRK5, GRK6) [6]. The rhodopsin kinase subfamily is primarily expressed in the retina and regulates opsins [7]. GRKs 2, 3, 5 and 6 are expressed ubiquitously and are known to play a role in GPCR phosphorylation in the cardiovascular system [8]. Alternatively, GRK4 is found primarily in the testes [9].

GRKs exhibit a tri-domain structure with a strongly conserved, centrally located catalytic domain (Fig. 2). Approximately, the first 30 N-terminal amino acids are highly conserved within the GRK superfamily and are necessary for recognition of the activated GPCR [10]. The N-terminal third also contains a regulator of G protein signaling (RGS) homology (RH) domain. It has been demonstrated that the RH domain of GRK2 and GRK3 is able to interact with $G\alpha_q$, while the RH domain of GRK5 and GRK6 has not been shown to interact with G-proteins [11,12].

The carboxyl (C)-terminal third of GRKs is responsible for membrane localization and is divergent between GRK subfamilies [13]. The ocular GRKs, 1 and 7, contain a short sequence which can be prenylated and this lipid group is responsible for interacting with the plasma membrane. Alternatively, GRK2 and 3 contain a C-terminal domain that binds to free Gβγ subunits after receptor activation [14]. This domain overlaps with a pleckstrin homology (PH) domain, which interacts with membrane phospholipids after Gβγ binding [14]. Meanwhile, GRK4, 5 and 6 cellular localization is mediated by the interaction with phosphatidylinositol 4, 5 bisphosphate (PIP2) within the plasma membrane [15]. GRK4 and GRK6 contain a palmitoylation site that does this while GRK5 is dependent on a positively charged lipid-binding element in the form of an amphipathic helix for membrane association [16,17].

1.3. The role of GRKs in the heart

The first GRK identified in the heart was GRK2, originally named βARK (or βARK 1) since it was purified with the β2-adrenergic receptor (AR) and found to phosphorylate this GPCR as well as other receptors [18]. βARK2, now known as GRK3, was then cloned and found to be very highly homologous to GRK2 with a very similar tissue distribution; however, expression levels of GRK3 were found to be approximately 10–20% that of GRK2 [19]. GRK5 was later discovered and found to be most highly expressed in the heart and muscle but it is also ubiquitously expressed [20,21]. Finally, GRK6, which shares a 70% amino acid identity with GRK5, was found to be expressed in the heart only weakly, but with greater expression in tissues such as the brain and skeletal muscle [22].

The importance of GRKs in the heart and in particular during cardiac development has been shown via genetic deletion in animal models. For example, GRK2 homozygous knockout (KO) is lethal in mouse models due to its role in embryonic development of the heart [23,24]. These animals die by gestational day 15.5, presumably due to heart failure (HF), as they exhibit hypoplasia of the ventricular myocardium and a 70% decrease in ejection fraction [23]. However, the embryonic lethality of GRK2 KO mice is not cardiomyocyte autonomous as early myocyte deletion does not lead to an abnormal heart or early death [24]. Conversely, the GRK5 homozygous KO mouse is viable, and no adverse effects have been attributed to this gene deletion [9]. GRK5 has been found to fine tune cardiac development in the zebrafish through the mTOR pathway. Zebrafish lacking the GRK5 homologue Grk5l develop hearts with incorrect symmetry [25]. Further, this study demonstrated for the first time embryonic lethality in GRK5/GRK6 double KO mice [25]. Previously, it was assumed that GRK5 was dispensable for embryonic development; however, this finding suggested that GRK6 is able to compensate for the loss of GRK5 in the mouse embryo [25].

As the heart transitions to disease, various cellular processes become dysregulated leading to diminished cardiac function that usually occurs after significant myocyte cell death. The failing heart (both human and animal models) has certain consistent molecular characteristics including desensitization and down-regulation of β-ARs, dysregulation of calcium (Ca^{2+}) handling and activation of the fetal gene program leading to the transcription of hypertrophic genes [26]. In health, catecholamines (i.e. norepinephrine and epinephrine) produced by sympathetic nervous system (SNS) neurons and the adrenal medulla act on α- and β-ARs in the heart to maintain homeostatic growth, function, and contractility. Sympathetic catecholamines can rapidly increase the rate and force of myocardial contractions in response to stress and exercise in the so-called "fight-or-flight" response. This crucial homeostatic process becomes abnormal in HF as the SNS is activated after cardiac injury as it senses a loss of cardiac output. However, over time augmented catecholamine production causes the desensitization and down-regulation of β-ARs through induction of GRK2 expression leading to insensitivity to the flight-or-flight response (Fig. 3A). This creates and perpetuates the vicious cycle of neurohormonal bombardment of the injured heart that cause increased cell death and further HF progression [27]. GRK5 is also up-regulated in the failing heart, and GRK's appear to play a crucial role in deconstruction of the fight-or-flight response in HF [28].

The loss of inotropic reserve in HF was the impetus for the use of β-AR agonist therapy. Although this strategy can be effective acutely, chronic administration has been shown to increase mortality [29]. Somewhat paradoxically, β-AR antagonists are the first line therapy for HF patients since they block the aforementioned catecholamine bombardment and rescue myocytes from the noxious effects of these neurohormones [30]. Although β-blockers reduce hospitalization and improve HF care, they leave a lot to be desired with respective to effective reversal of disease; further, animal models show only modest effects to increasing failing heart function [31–33]. Thus, new therapies are needed and data from our lab has shown that GRK inhibition appears to be a novel therapeutic strategy capable of restoring the fight-or-flight response, inducing enhanced cardiac function, and reversal of disease in various ani-