Journal of Molecular and Cellular Cardiology 50 (2011) 785–792

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

GRK2 as a novel gene therapy target in heart failure

Giuseppe Rengo [a,b,d], Anastasios Lymperopoulos [a,c], Dario Leosco [d], Walter J. Koch [a,*]

[a] Center for Translational Medicine and George Zallie and Family Laboratory for Cardiovascular Gene Therapy, Department of Medicine, Thomas Jefferson University, Philadelphia, PA, USA
[b] Department of Cardiology, IRCCS Fondazione Salvatore Maugeri, Telese Terme (BN), Italy
[c] Department of Pharmaceutical Sciences, Nova Southeastern University College of Pharmacy, Ft Lauderdale, FL, USA
[d] Department of Clinical Medicine, Cardiovascular and Immunological Sciences, University of Naples "Federico II", Naples, Italy

ARTICLE INFO

Article history:
Received 28 June 2010
Received in revised form 16 August 2010
Accepted 17 August 2010
Available online 25 August 2010

Keywords:
β-adrenergic receptor
Heart failure
Gene therapy
G protein-coupled receptor kinase
βARKct
Neurohormonal feedback

ABSTRACT

Despite significant advances in pharmacological and clinical treatment, heart failure (HF) remains a leading cause of morbidity and mortality worldwide. HF is a chronic and progressive clinical syndrome characterized by a reduction in left ventricular (LV) ejection fraction and adverse remodeling of the myocardium. The past several years have seen remarkable progress using animal models in unraveling the cellular and molecular mechanisms underlying HF pathogenesis and progression. These studies have revealed potentially novel therapeutic targets/strategies. The application of cardiac gene transfer, which allows for the manipulation of targets in cardiomyocytes, appears to be a promising therapeutic tool in HF. β-adrenergic receptor (βAR) dysfunction represents a hallmark abnormality of chronic HF, and increased G protein-coupled receptor kinase 2 (GRK2) levels/activity in failing myocardium is among these alterations. In the past 15 years, several animal studies have shown that expression of a peptide inhibitor of GRK2 (βARKct) can improve the contractile function of failing myocardium including promoting reverse remodeling of the LV. Therefore, data support the use of the βARKct as a promising candidate for therapeutic application in human HF. Importantly, recent studies in cardiac-specific GRK2 knockout mice have corroborated GRK2 being pathological in failing myocytes. The purpose of this review is to discuss: 1) the alterations of βAR signaling that occur in HF, 2) the evidence from transgenic mouse studies investigating the impact of GRK2 manipulation in failing myocardium, 3) the therapeutic efficacy of *in vivo* βARKct gene therapy in HF, and 4) the intriguing possibility of lowering HF-related sympathetic nervous system hyperactivity by inhibiting GRK2 activity in the adrenal gland. This article is part of a Special Section entitled "Special Section: Cardiovascular Gene Therapy".

Contents

1. Introduction

Heart failure (HF) represents the common end of many different forms of heart disease and is a pathological condition due to the inability of the heart to fill with or eject blood adequately. It represents the ultimate outcome of several different disease conditions including coronary artery disease, hypertension, and viral or

* Corresponding author. Center for Translational Medicine, Thomas Jefferson University, 1025 Walnut St, Room 317, Philadelphia, PA 19107, USA.
E-mail address: walter.koch@jefferson.edu (W.J. Koch).

0022-2828/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2010.08.014

congenital cardiomyopathy. Although there have been improvements in therapy, HF still represents one of the most common public health problems worldwide [1]. Unfortunately, current medical treatments, including angiotensin-converting enzyme (ACE) inhibitors, sartans, diuretics and β-adrenergic receptor (βAR) blockers are only able to mitigate patient symptoms but fail to halt HF progression and to improve global cardiac function, thus they are far from ideal [1]. Importantly, increased understanding of the molecular pathogenesis of HF is leading to the identification of new entities that could serve as future therapeutic targets. Of interest, some of these targets appear particularly amenable to the application of gene therapy. In fact a variety of catheter or surgical approaches to *in vivo* cardiac gene transfer in animal models have provided very promising results showing improvement of cardiac function and rescue of failing myocardium [2,3]. Indeed, these results coupled with studies in genetically engineered mice have validated several new targets for HF gene therapy and a few of these are at different stages of translational development [3].

An important fact that should not be overlooked is that there are three ongoing human HF gene therapy clinical trials, two of which are targeting sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA2a) [4,5]. A third trial targets overexpression of adenylyl cyclase (AC) Type VI [6,7] (see http://clinicaltrials.gov/show/NCT00787059). All other potential candidate molecules for future gene therapy application are at a pre-clinical stage of investigation. Among these latter molecules, promising results have been obtained with gene delivery interventions targeting proteins involved in cardiomyocyte calcium (Ca^{2+}) handling (phospholamban [8], protein phosphatase 1 inhibitor [9], parvalbumin [10] and S100A1 [11]), or targeting G protein-coupled receptor (GPCR) kinase-2 (GRK2) [12], the subject of this review.

Failing myocardium is characterized by alterations in β-adrenergic receptor (βAR) signaling due, at least in part, to increased GRK2 levels/activity [13,14]. Over the past two decades several experimental studies have shown that limiting βAR down-regulation/desensitization via GRK2 inhibition in HF is therapeutic [15]. In addition, GRK2 inhibition, no doubt, also blocks desensitization of several other G protein-coupled receptor (GPCR) systems that may also contribute to the effects in the myocytes seen in studies described below [15]. This review will focus on the therapeutic effects of GRK2 inhibition by gene therapy in HF using a peptide derived from the carboxyl terminus of GRK2 known as the βARKct. The βARKct displaces endogenous GRK2 from the membrane and prevents desensitization of GPCRs. Moreover, we will discuss the fascinating possibility to lower HF-related sympathetic nervous system (SNS) hyperactivity by inhibiting GRK2 activity specifically in the adrenal gland.

2. SNS hyperactivity and cardiac βAR dysfunction in HF: role of GRK2

Although GRK2 phosphorylates several GPCRs in the heart and there is little doubt that its inhibition affects signaling through multiple receptor systems, we focus on βARs since derangements in this system in HF are central to the experiments leading to identification of GRK2 as a therapeutic target. βARs are typical GPCRs that, following agonist binding, activate heterotrimeric G-proteins [15]. The principal role of βARs in the heart is the regulation of cardiac rate and myocyte contractile force in response to the SNS catecholamine (CA) neurotransmitters, epinephrine (Epi) and norepinephrine (NE). βARs are comprised of three subtypes β_1, β_2 and β_3 ARs, each one with its own functional and molecular properties. β_1ARs are the predominant subtype in the myocardium, representing 75–80% of total βAR density, followed by β_2AR, which compose about 20% of total receptors (under normal conditions), while the β_3AR is present in minor amounts [16]. β_1AR stimulation by CAs results in the dissociation of the stimulatory G protein α-subunit ($G\alpha_s$) from $G_{\beta\gamma}$, $G\alpha_s$ stimulates AC to produce cAMP, which by activating protein kinase A (PKA) regulates different intracellular, sarcolemmal and myofibrillar substrates, thus exerting the cellular effects of receptor activation on cardiac chronotropy, inotropy and lusitropy (Fig. 1(A)) [17]. In addition, $G_{\beta\gamma}$ can also activate down-stream effectors that participate in cardiac signaling [18]. β_2ARs also mediate the effects of CAs on the heart, but in a qualitatively different manner from β_1ARs, as they can also couple to the AC inhibitory G protein (Gi) [15,19]. This dual Gs/Gi coupling has been implicated in differential β_2AR signaling specifically concerning myocyte apoptosis as β_2AR-Gi is cardioprotective, while this doesn't occur with the pro-apoptotic β_1ARs [19–21].

Following cardiac stress/injury several neurohormonal systems are hyperactive; in particular, elevated SNS activity and outflow occurs with increased circulating levels of Epi and NE [15,22]. It is widely recognized that chronic stimulation of the β adrenergic system by these CAs exerts toxic effects on the heart and plays a key pathogenic role in HF progression [15,22]. Indeed, clinical administration of βAR agonists, despite producing immediate hemodynamic benefits, reduces the overall survival of patients with chronic HF [23]. Alternatively, success of β-blocker treatment at reducing HF progression and related morbidity and mortality appears primarily attributable to the ability of these drugs to protect the heart from the detrimental effects of elevated CAs [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 failing human heart for the first time [25]. 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 (Fig. 1(B)) [16,17]. βAR dysfunction is characterized at the molecular level by selective reduction of β_1AR density at the plasma membrane (down-regulation) and by uncoupling of the remaining membrane β_1ARs and β_2ARs from G-proteins (desensitization) [14,17,22].

The abnormal desensitization of βARs in injured and failing myocardium appears to be mediated by two GRKs, GRK2 and GRK5, which have both been shown to be elevated in HF models [14,26,27] and in human disease [13,28]. Most of the HF data regarding GRKs is focused on GRK2, which is the subject of this review. However, it is interesting to note that we have recently shown that elevated GRK5 can lead to pathological hypertrophy due to novel activity in the nucleus of myocytes [29]. Increased interest into the role that GRK2 plays in cardiovascular pathophysiology is bolstered by the fact that this kinase is up-regulated in several different pathologic conditions, such as cardiac ischemia [30], hypertrophy [31], and hypertension [32]. In HF, cardiac GRK2 protein levels are elevated in the early stages of the disease and several lines of evidence suggest levels of this kinase can serve as a potential novel biomarker of cardiac dysfunction in human HF [33–35]. Currently, the general consensus is that the excessive amount of SNS activity and CAs 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 [22]. As described in more detail below, GRK2 activity following cardiac stress/injury is detrimental to the heart and this appears to involve βARs although it is probable that other GPCR systems are also involved.

3. Role of GRK2 and its inhibition in the normal and failing heart: lessons from studies in genetically engineered mice

GRK2 is a serine/threonine kinase within a family of seven members (GRK1–7). All GRKs share a common structural architecture with a well-conserved, central catalytic domain (~270 aa), similar to that of other serine/threonine kinases, flanked by an amino-terminal (NT) domain (~185 aa) and a variable-length carboxyl-terminal (CT) domain (~105–230 aa) that contains specific regulatory sites [36,37]. Several of these GRKs are ubiquitously expressed, including in the