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GRK2 as a Novel Gene Therapy Target in Heart Failure

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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 unravelling 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 (β AR) 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 (β ARKct) can improve the contractile function of failing myocardium including promoting reverse remodeling of the LV. Therefore, data support the use of the β ARKct 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 β AR signalling 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* β ARKct 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.

Keywords

β -adrenergic receptor; heart failure; gene therapy; G protein-coupled receptor kinase; β ARKct neurohormonal feedback

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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 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 (β AR) 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 preclinical 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 (β AR) 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 β AR 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 β ARKct. The β ARKct 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.

SNS hyperactivity and cardiac β AR 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 β ARs since derangements in this system in HF are central to the experiments leading to identification of GRK2 as a therapeutic target. β ARs are typical GPCRs that, following agonist binding, activate heterotrimeric G-proteins [15]. The principal role of β ARs 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). β ARs

are comprised of three subtypes β_1 , β_2 and β_3 ARs, each one with its own functional and molecular properties. β_1 ARs are the predominant subtype in the myocardium, representing 75–80% of total β AR density, followed by β_2 AR, which compose about 20% of total receptors (under normal conditions), while the β_3 AR is present in minor amounts [16]. β_1 AR stimulation by CAs results in the dissociation of the stimulatory G protein α -subunit (G_{α_s}) from $G_{\beta\gamma}$, G_{α_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 (Figure 1A) [17]. In addition, $G_{\beta\gamma}$ can also activate downstream effectors that participate in cardiac signaling [18]. β_2 ARs also mediate the effects of CAs on the heart, but in a qualitatively different manner from β_1 ARs, as they can also couple to the AC inhibitory G protein (G_i) [15,19]. This dual Gs/Gi coupling has been implicated in differential β_2 AR signaling specifically concerning myocyte apoptosis as β_2 AR-Gi is cardioprotective, while this doesn't occur with the pro-apoptotic β_1 ARs [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 β AR 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 β AR 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 β AR system that take place during HF development, and it is now well known that the chronically elevated CA stimulation causes significant derangements of β AR signaling and function in HF (Figure 1B) [16,17]. β AR dysfunction is characterized at the molecular level by selective reduction of β_1 AR density at the plasma membrane (down-regulation) and by uncoupling of the remaining membrane β_1 ARs and β_2 ARs from G proteins (desensitization) [14,17,22].

The abnormal desensitization of β ARs 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 β AR 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 β ARs although it is probable that other GPCR systems are also involved.

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 heart where GRK2 represents the most abundant isoform [14]. GRK2 has the property that as a primarily cytosolic protein it must become membrane-associated in order to come in contact and phosphorylate agonist-occupied GPCRs [36,37]. It has been shown that for GRK2 this occurs through binding sites within its CT domain and membrane-embedded $G_{\beta\gamma}$ subunits that are free to bind to GRK2 only after GPCR activation [36,37]. In the heart, CA activation of β ARs induces $G_{\beta\gamma}$ -mediated GRK2 translocation to initiate desensitization (Figure 1). Following phosphorylation of the receptor by GRKs, there is enhanced affinity of the receptor for binding to β -arrestins, which sterically prevents further G protein activation [37,38]. Further, β -arrestins elicit novel, G protein independent signals through scaffolding functions [37,38]. The membrane translocation of GRK2 via $G_{\beta\gamma}$ binding is the point of development for the β ARKct peptide, which competes with endogenous GRK2 for $G_{\beta\gamma}$ binding and effectively acts as a GRK2 inhibitor (Figure 1) [39].

It is apparent that initially, the up-regulation of GRK2 observed after cardiac stress/injury is a protective mechanism to defend the heart from the toxic effects of excessive CA stimulation by reducing signaling through β ARs. Indeed, chronic administration of β AR agonists increase expression of GRK2 in the heart and reciprocally, β -blocker treatment decrease cardiac GRK2 levels [40]. Our hypothesis has always been that over time, this specific GRK2-dependent mechanism of blunting β AR signaling becomes maladaptive as it feeds into the vicious pathological cycle of increasing SNS activity since β ARs are not responding to stimulation [22]. Fifteen years ago, we began to investigate the role of GRK2 in the heart and found that when GRK2 is overexpressed specifically in cardiomyocytes of transgenic mice to the level of up-regulation found in human HF (3–4 fold), *in vivo* β AR signaling and contractile reserve was blunted showing that indeed this GRK could functionally uncouple endogenous receptors [38]. Contractile response to angiotensin II stimulation was also blunted in these mice suggesting that indeed, GRK2 is targeting other receptor systems in the heart [41].

In order to begin studying potential effects *in vivo* in the heart to GRK2 inhibition, cardiac-specific β ARKct transgenic mice were generated in order to prevent $G_{\beta\gamma}$ -mediated membrane translocation/activation [38] (see Figure 1C). Interestingly, β ARKct expression in the heart enhanced cardiac contractility both at baseline and after adrenergic stimulation [38]. Moreover, in a second study, cardiac β ARKct expression was able to reverse the contractile and β AR dysfunction due to transgenic overexpression of GRK2 [42]. These results demonstrate 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.

Following these initial characterization studies of transgenic β ARKct expression, the hypothesis that myocardial GRK2 elevation during disease progression is maladaptive was tested using genetic mouse models of HF. The first model tested was the muscle LIM-domain protein (MLP) knockout (KO) mice and when these mice were bred with cardiac β ARKct transgenic mice there was prevention of the dilated cardiomyopathic phenotype [43]. The mice failed to progress to HF and there was restoration of cardiac contractile reserve and β AR signaling [43]. Similarly positive results were obtained when HF mice

overexpressing the Ca^{2+} binding protein calsequestrin (CSQ) were crossed with the β ARKct line [44]. In this case GRK2 inhibition not only improved cardiac contractility with positive hemodynamic effects, but also nearly doubled the life-span of these mice. Moreover, this study demonstrated for the first time the synergistic effects of β ARKct gene therapy with β -blockers on survival in HF [44]. In addition, in a mouse model hypertrophic cardiomyopathy, transgenic β ARKct expression prevented cardiac hypertrophy and improved exercise tolerance along with hemodynamic benefits [45]. Not every HF mouse model was rescued by β ARKct expression and there appeared to be some model dependency as GRK2 inhibition did not prevent cardiomyopathy in $\text{G}\alpha_q$ -overexpressing transgenic mice [46] or mice with overexpression of a mutant CREB transcription factor [47].

Overall, more positive effects were seen than neutral effects and HF rescue by β ARKct in mouse models provided powerful evidence that GRK2 inhibition could result in a normalization of β AR signaling and improvement of functional and morphological parameters. Since the β ARKct works through a $\text{G}_{\beta\gamma}$ -GRK2 inhibitory fashion, the ultimate determination of whether GRK2 is pathological in HF progression needed to be done in a mouse KO model. This became problematic when classical GRK2 gene deletion was found to be embryonic lethal [48]. However, consistent with β ARKct mice, heterozygous GRK2 KO mice, which develop normally and had 50% expression levels of GRK2 in the heart, presented with increased cardiac function and improved β AR-mediated inotropic reserve similar to that found in β ARKct mice [49]. More recently, by taking advantage of the availability of conditional GRK2 KO mice [50], the critical role of GRK2 in HF pathogenesis and progression has been confirmed [51]. In this study, mice with GRK2 knocked down specifically in cardiomyocytes from birth underwent myocardial infarction (MI) by surgical ligation of the left anterior descending coronary artery, and HF progression was prevented even with a large infarct present [51]. Interestingly, these mice also lacked the characteristic β AR signaling abnormalities present in control post-MI mice. In a second set of experiments, induced GRK2 gene ablation in myocytes was done at 10 days post-MI and this prevented subsequent post-MI death and reversed pathological LV remodeling and actually increased cardiac function [51]. Metoprolol was also administered in a cohort of mice and GRK2 KO proved more beneficial than β AR blockade in this model [51].

Taken together, the results from the above studies using β ARKct transgenic mice as well as cardiac-specific GRK2 KO mice, provide compelling evidence for enhanced GRK2 expression observed in HF being detrimental for the heart and strongly support the notion that cardiac GRK2 inhibition using the β ARKct might be a valid gene therapy therapeutic strategy. Certainly, there is considerable overlap between HF rescue with β ARKct and GRK2 KO but that doesn't eliminate the possibility that the β ARKct may be doing more (via $\text{G}_{\beta\gamma}$ inhibition) than blocking GRK2 activity. Additive mechanisms of β ARKct continue to be an area of exploration of our lab.

Gene therapy in HF: general considerations

From a practical point of view, the *in vivo* success of cardiac gene therapy depends on the tissue-selectivity of the approach for the myocardium and the reduction of potential adverse effects. These aspects are strictly linked to two main technical features in cardiac gene therapy: the choice of the vehicle/vector and the choice of the gene delivery technique to carry the therapeutic gene into the failing myocardium *in vivo*. In this review, we will only briefly touch on these aspects since they are more extensively discussed in other review articles within this series.

Gene delivery vehicles include a variety of viral and non-viral vectors. Overall, viral vectors are more efficient than non-viral in terms of myocardial transduction and long-term cardiac expression, but unfortunately they are not free of significant side-effects. Adenoviral

(Ad) vectors have been widely used in the vast majority of gene therapy studies in heart, since these vectors efficiently deliver and express genes in the myocardium. However, the main disadvantage of Ad vectors is that they encode immunogenic viral proteins that trigger cytotoxic immune responses *in vivo* leading to the elimination of the cells infected resulting in transient transgene expression. Alternatively, adeno-associated virus (AAVs) vectors seem to be more promising for *in vivo* myocardial gene therapy, since these vectors are largely non-immunogenic, produce stable and long-term transgene expression including in the heart, and have been safely utilized in gene therapy studies in animals as well as in humans. Conventional AAV vectors derived from AAV serotype 2 with a broad tissue tropism are usually preferred, but some serotypes have been shown to exert higher tropism for cardiac tissue [52,53]. The main drawbacks of these vectors are their low packaging capability, the presence of antibodies against some viral epitopes in the human population, and importantly, the fact that very little is known about their potential long-term *in vivo* adverse effects.

Gene delivery methods—Over the past years, several gene delivery techniques have been proposed to achieve transgene expression in the whole myocardium; however, most of these have been conducted in animal models of heart disease. Potentially, a single vein injection of vector represents the ideal method to transduce myocardium *in vivo*. This method has been successfully applied in small animals, but its translational application to larger animals and humans is probably not feasible because of the need higher doses of vectors in order to sufficiently infect cardiac muscle. In the context of a more generalized disease of the myocardium such as HF, a technique that supports global heart delivery would be desirable. Direct intra-myocardial injection of viral particles has been utilized in several experimental animal models, including large animals, with good results and it appears to be a good candidate for human gene transfer, in particular for those patients undergoing procedures of cardiothoracic surgery. Alternatively, delivery via intracoronary artery has also been tested in experimental HF studies, resulting in robust myocardial expression of the gene of interest. However, with this approach, additional agents such as substance P are required to pass the endothelial barrier and achieve significant viral transfer to the myocardium. Another limitation of intracoronary delivery is that the single passage of the vector through the coronary circulation provides short time for gene uptake from the heart. A variety of approaches have been proposed to improve the efficacy of this technique, such as use of a high pressure delivery method with proximal balloon occlusion, stoppage of the heartbeat during gene transfer or retrograde delivery via the coronary veins. Nevertheless, and despite some of the above limitations, antegrade coronary artery injection of AAV1 is being employed in a HF gene therapy clinical trial [4,5].

Targeting GRK2 by gene therapy in HF

As covered by this review series, gene therapy approaches for cardiovascular diseases appear to be a very attractive and promising tool for translating the results from experimental studies in transgenic mice into clinical practice. In fact, translation from animal models to humans is progressing with HF taking the lead following early work in angiogenesis. To begin to test whether β ARKct-mediated GRK2 inhibition could be done *in vivo* outside of the mouse via gene transfer, we initially cloned it into an adenoviral (Ad) vector. *In vitro* testing of Ad- β ARKct found that this could indeed potentiate β AR signaling in adult rabbit ventricular myocytes [54] as well as reverse dysfunction of β ARs in failing rabbit ventricular myocytes [55]. *In vitro* gene transfer of Ad- β ARKct to failing human myocytes also induced improvement of contractile dysfunction and β -adrenergic responsiveness providing proof of concept for the feasibility of this approach in human HF [56].

Having proven the therapeutic efficacy of GRK2 inhibition in myocytes, the next step was to validate its applicability in *in vivo* gene therapy animal studies. GRK2 inhibition was first tested in rabbits in a study where Ad- β ARKct was administered into the coronaries at the time of MI [57]. Three weeks post-gene transfer, GRK2 inhibition resulted in prevention of LV adverse remodeling, improvement of cardiac contractility, and preservation of β AR signaling and function [57]. This study did not only represent the first *in vivo* proof of β ARKct's therapeutic benefit in HF, but also demonstrates that β AR down-regulation and desensitization are maladaptive mechanisms that need to be antagonized to attain therapeutic benefit. A second study investigated HF rescue with Ad- β ARKct being delivered to rabbit hearts 3 weeks post-MI, a time at which the signs of HF were already evident [58]. One week post-gene transfer, β ARKct expression resulted in improvement of LV dysfunction, reduction of LV end diastolic pressure and reversal of β AR desensitization [58]. The results of these two studies clearly show that GRK2 inhibition has therapeutic potential interfering both with the establishment and the progression of post-ischemic HF.

In other studies, β ARKct was expressed in the right ventricle (RV) of rabbits in a model of RV failure induced by pulmonary artery binding [59,60]. In this model a β ARKct-dependent amelioration of RV function was observed and, importantly, improvement of survival. Two further studies tested β ARKct gene transfer in the setting of acute myocardial injury [61,62]. The alterations of β AR signaling observed in HF, including GRK2 overexpression, are also manifest in response to acute stress, thus GRK2 inhibition also proved to be therapeutic in a clinically relevant model of LV dysfunction following cardioplegic arrest, positively influencing LV and β AR function [61,62].

The above studies were limited to a more acute window of observation due to the limitations of Ad vectors that include only short-term expression and high inflammatory responses *in vivo* [63]. The chronic nature of HF necessitates testing the long-term effects of β ARKct gene therapy in order to demonstrate its true translational impact and thus, the β ARKct was cloned into adeno-associated viruses (AAVs). AAV vectors are more promising for *in vivo* myocardial gene therapy since they are largely non-immunogenic, produce stable and long-term transgene expression including in the heart, and have been safely utilized in gene therapy studies in animals as well as in humans [2]. Of note, conventional AAV vectors derived from AAV serotype 2 with a broad tissue tropism are usually preferred, but some serotypes have been shown to exert higher tropism for cardiac tissue [52,53].

We have recently published the first chronic β ARKct gene therapy study in a rat model of HF [12]. In this study, HF was induced by a LV cryo-infarct and after 12 weeks, *in vivo* cardiac function was assessed to ensure HF was established. Rats were then randomly assigned to different treatment groups and AAV6- β ARKct was administered via a direct intramyocardial injection [12]. Control groups included rats undergoing a sham procedure as well as HF rats treated with saline and AAV6-GFP (green fluorescent protein). We also treated a group of GFP-HF rats and β ARKct-HF rats with the β_1 AR antagonist, metoprolol [12]. Twelve weeks post-gene transfer, sustained β ARKct expression throughout the LV resulted in reversal of pre-existing HF as demonstrated by the improvement of LV ejection fraction and remodeling [12]. Further, at the molecular level, HF-related β AR dysfunction was completely reversed with a restoration in cardiac β AR density at the plasma membrane, cAMP production and GRK2 expression [12]. Importantly, GRK2 inhibition by gene therapy and β -blocker treatment were completely compatible, in fact, all the beneficial effects of β ARKct were preserved in association with metoprolol, adding to the potential future introduction of β ARKct gene therapy in clinical HF treatment. Moreover, the molecular effects of β AR blockade (metoprolol) and of GRK2 inhibition were similarly beneficial in the long run in that both therapeutic modalities were able to lower GRK2 levels in the heart, preserve functional β AR numbers at the plasma membrane and reverse β AR

uncoupling from G-proteins, with normalization of myocyte cAMP content [12], which is a primary molecular determinant of cardiac contractility (Figure 1D). Interestingly, only β ARKct (with or without metoprolol) positively improved cardiac performance in the failing heart suggesting potential additional mechanisms of beneficial action beyond β AR signaling. Finally, this study proposed, as a contributing mechanism for the beneficial effects of long-term GRK2 inhibition and metoprolol treatments, the restoration of HF-related neurohormonal status; in fact, both treatments resulted in normalization of the circulating CA and aldosterone levels, which decreases the stress put on the failing heart [12].

Inhibition of adrenal GRK2 to counter HF-related SNS hyperactivity

As mentioned above, one of the most relevant features of HF is SNS hyperactivity and outflow, which is reflected by increased levels of circulating CAs Epi and NE [15]. Initially an adaptive mechanism aiming to compensate decreased contractility following cardiac injury, it ultimately becomes maladaptive, contributing to HF establishment and progression [22]. Circulating CAs are derived from two main sources in the body: sympathetic nerve terminals, releasing NE, and the adrenal medulla, secreting mainly Epi. 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_2 ARs, acting as presynaptic inhibitory autoreceptors [22]. α_2 ARs, as many other GPCRs, undergo agonist-induced desensitization via GRKs and subsequent down-regulation [64]. Of note, there is an increased GRK2 expression and activity in the adrenal medulla, which critically influences CA secretion from this source [65]. In particular, we have shown that in an animal model of HF, adrenal GRK2 overexpression is responsible for α_2 AR dysfunction, thus the inhibitory presynaptic function of this receptor is lost - resulting in chronically elevated CA secretion [65]. This apparent crucial role for adrenal GRK2 in HF is underlined by the fact that we found that its specific inhibition, via Ad-mediated β ARKct adrenal gene delivery, leads to a significant reduction in CA circulating levels, “resetting” not only adrenal, but also cardiac function [65]. In fact, post-MI, HF rats treated with intra-adrenal β ARKct gene delivery showed improved myocardial contractility and β AR signaling [65]. The results of this study provide strong evidence for significant crosstalk at the level of entire organs in a complex syndrome such as HF. Further, it identified adrenal GRK2 as a potential sympatholytic target for HF treatment, through the restoration of α_2 AR function in the adrenal medulla and its consequent inhibition of CA secretion from this source [65]. Most recently, we used Epi-producing cell specific GRK2 KO mice to show that indeed, with limited GRK2 expression, there is less CA secretion post-MI and the resultant decreased circulating CAs improved cardiac function and β AR reserve [66].

Adrenal GRK2 has also been shown to critically modulate CA secretion under normal conditions. In fact, in normal healthy rats, adrenal β ARKct gene transfer resulted in reduced plasma circulating CA levels; in contrast, GRK2 overexpression in the adrenal medulla led to increased Epi and NEpi plasma levels [67]. To increase the importance of adrenal GRK2, we have recently shown that exercise training, which exerts several beneficial effects on cardiovascular system including curbing HF-related SNS hyperactivity, is able to normalize GRK2 expression and restore α_2 AR function in the adrenal glands of HF rats [68].

It is quite plausible that, during HF, GRK2-dependent α_2 AR 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 these studies propose GRK2 inhibition as a new sympatholytic strategy in HF, blocking CA release at the sources of these hormones preventing their toxic effects on peripheral organs, like the heart. In addition, adrenal β ARKct 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 extra-cardiac effects of CAs, including the activation of the endothelin and the renin-angiotensin-aldosterone systems. Moreover, adrenal GRK2 inhibition might allow for reduction of β -blocker dosage and adverse effects in HF.

Summary and Future Therapeutic Considerations

Despite extensive research, gene therapy and in particular cardiac gene therapy for HF, are still in their infancy. However, the vast plethora of data produced over the last 15 years on the therapeutic efficacy of GRK2 inhibition and more recently with conditional GRK2 KO mice (summarized in Table 1) make the β ARKct a promising candidate for future application in human HF. A note of caution exists since the true therapeutic potential of GRK2 inhibition via β ARKct gene delivery needs further validation in pre-clinical large animal studies before being considered for use in the therapeutic armamentarium for human HF. Importantly, a large animal HF study using AAV- β ARKct is underway with the goal of a Phase I clinical trial in the next couple of years. Mechanistically, it appears that β ARKct works, at least in part, via normalization of β AR signaling (Figure 1), however, this does not eliminate other contributing mechanisms that appear likely as well as $G_{\beta\gamma}$ inhibition or resensitization of other GPCRs. However, since human application faces many hurdles, including potential long-term vector adverse effects, eventual development of advanced generation vectors and improvement of the gene delivery techniques to reach highly cardio-selective transgene expression are warranted. Further, before a human application of β ARKct gene therapy occurs it will be necessary to evaluate this potential therapeutic advance in a pre-clinical large animal model of HF. Included in these studies will be the need to test the effect on cardiac function via a dose-dependency; the presence of anti-AAV antibodies; its safety and toxicological profile, including potential organ damage and inflammatory responses and, its effects on cardiac energetics and arrhythmias; its compatibility with established HF-therapies (such as ACE-inhibitors and β -blockers). Alternatively, there are several ongoing efforts to develop pharmacologic inhibitors of GRK2, which would presumably avoid all these issues related to gene therapy application and safety in humans.

Finally, as detailed above, simultaneous inhibition of GRK2 in both the heart and the adrenal gland appears ideally suited to combat both the toxic effects of elevated CA levels and the decline in contractility of the failing heart, thus globally restoring cardiac function/status in HF.

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Figure 1. Cardiomyocyte β AR signaling (A) under normal physiological condition, (B) during heart failure, and the positive effects on β AR function of (C) GRK2 inhibition (via β ARKct) and (D) β -blocker treatment. The figure denotes that both β AR blockade and β ARKct expression resensitizes β ARs and promotes normalization of cAMP signaling. Notably, other downstream mechanistic effects of β ARKct expression could also occur including resensitization of other GPCRs as well as inhibition of other $G_{\beta\gamma}$ -mediated signaling pathways.

Table 1

Animal models supporting the therapeutic value of GRK2 inhibition

Model	Therapeutic strategy	Molecular outcome	Functional/Phenotypic outcome	Reference
Transgenic mouse models				
GRK2 homozygous KO	NA	Embryonic death		48
Cardioselective GRK2 overexpression	NA	β AR desensitization; reduced AC activity	Reduced ISO-stimulated cardiac contractility	40
Cardioselective β ARKct expression	β ARKct	NI	Enhanced basal and ISO-stimulated cardiac contractility	40
Concomitant cardioselective GRK2 and β ARKct expression	β ARKct	Normalized GRK2 activity and β AR density	Restored cardiac contractility	41
GRK2 heterozygous KO	Reduced (50%) GRK2 expression	NI	Enhanced basal and ISO-stimulated cardiac contractility	42
GRK2 heterozygous KO and cardioselective β ARKct expression	Reduced (50%) GRK2 expression + β ARKct	NI	Enhanced basal and ISO-stimulated cardiac contractility	42
MLP KO	β ARKct	Reduced GRK2 expression; increased β AR density and AC activity	Normalized LV diameters and improved basal and ISO-stimulated cardiac contractility	43
CSQ Cardioselective overexpression	β ARKct with or without β -blocker	Reduced GRK2 expression; increased β AR density	Increased survival; reduced LV diameters and increased cardiac contractility	44
Cardioselective GRK2 KO + MI	Reduced GRK2 expression	NI	Increased survival; reduced ventricular diameters and increased cardiac contractility	51
Post-MI conditional GRK2 KO	Reduced GRK2 expression 10 days after MI	NI	Increased survival; reduced ventricular diameters and increased cardiac contractility	51
Viral-mediated gene therapy: <i>in vitro</i> studies				
Cultured adult rabbit ventricular myocytes	β ARKct	Increased β AR density	Enhanced basal and ISO-stimulated myocyte contractility	52
Cultured failing rabbit ventricular myocytes	β ARKct	Reduced GRK2 activity; increased β AR density and AC activity	NI	53
Failing human ventricular myocytes	β ARKct	Increased AC activity	Enhanced ISO-stimulated myocytes contractility	54
Viral-mediated gene therapy: <i>in vivo</i> studies				
Gene delivery of Ad β ARKct at the time of MI in rabbit	β ARKct	Reduced GRK2 expression; increased β AR density and AC activity	Enhanced basal and ISO-stimulated cardiac contractility	55
Gene delivery of Ad β ARKct 3 weeks post-MI in rabbit	β ARKct	Enhanced AC activity	Enhanced cardiac contractility	56
Gene delivery of Ad β ARKct to the RV 3 days before pulmonary artery banding	β ARKct	Increased AC activity	Attenuation of RV dilatation and dysfunction	57

Model	Therapeutic strategy	Molecular outcome	Functional/Phenotypic outcome	Reference
Gene delivery of Ad β ARKct to the RV 3 days before pulmonary artery banding	β ARKct	NI	Increased survival	58
Cardioplegic arrest 5 days post-gene delivery of Ad β ARKct in rabbit	β ARKct	Normalized GRK2 levels/activity, β AR density and AC activity	Increased LV function	59
Gene delivery of Ad β ARKct at the time of MI in rabbit (acute effects)	β ARKct	Normalized GRK2 levels, β AR density and AC activity	Preserved cardiac contractility	60
Gene delivery of AAV6- β ARKct in HF rats (long-term effects)	β ARKct with or without β -blocker	Normalized GRK2 levels, β AR density, cAMP production and normalized CA and aldosterone levels	Reduced LV diameters, enhanced basal and ISO-stimulated cardiac contractility	12

NA: not applicable, NI: not investigated.