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Adrenergic Receptor Signaling Components in Gene Therapy

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Summary

Adrenergic receptor (AR) signaling is a key regulator of normal cardiopulmonary homeostasis. Under pathophysiological conditions, such as heart failure, asthma, and hypertension, there are alterations in the signaling cascades. Advances in the ability to manipulate the adenoviral genome have allowed the development of gene therapy in which transgenes of interest are inserted into the adenovirus and transferred to mammals in an organ-specific manner based on delivery methods. These transgenes have included components of the AR signaling pathway that have gone awry at the level of the AR itself or the G protein it activates, the G protein-coupled receptor kinases (GRKs), and regulators of G protein signaling (RGS) proteins that regulate AR desensitization, or the adenylyl cyclase that subsequently activates protein kinase A activity. The use of these vectors in both the heart and the lung has offered promising novel benefits for animal models of disease, including heart failure and lung disorders, and it remains to be determined whether these will be successful future therapeutic strategies in human disease.

Key Words: Adenovirus; adenylyl cyclase; β-adrenergic receptor signaling; G protein; G protein-coupled receptor kinase; heart failure; regulator of G protein signaling (RGS) protein.

1. Introduction

Adrenergic receptor (AR) signaling components are essential for the establishment and maintenance of overall homeostasis. Pathophysiologies and dis-

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ease states can arise when there are aberrations in the AR signaling cascades, and dysfunctional AR signaling can be associated with different disorders. This phenomenon has been best characterized in the cardiovascular and respiratory systems. A goal in the generation of novel therapeutics to treat heart failure, hypertension, and lung disorders has been either to augment or to attenuate abnormal adrenergic signaling cascades using gene therapy. Although not yet at the clinical stage, these methods have been extensively studied in animal models of human disease.

At present, gene therapy is primarily accomplished using adenoviral vectors *(1)*. The adenovirus used has been engineered such that it lacks an envelope and has a 36-kb double-stranded deoxyribonucleic acid (DNA) genome, and it is no longer capable of viral replication *(1)*. The virus is not integrated into host DNA, but rather it persists in the cell as episomal DNA. Adenovirus has produced robust transgene expression in cardiomyocytes, and it can easily be produced in quantities sufficient for experimentation. The advent of adenoviral-mediated gene transfer has provided researchers with a powerful tool to examine signaling pathways in animal models of disease, and it has the potential to provide clinicians with an effective new therapeutic tool.

2. Potential Gene Therapy Targets

2.1. Adrenergic Receptors

The signaling cascade activated with AR stimulation is similar between the three major subclasses of ARs: α_1, α_2, and β (Fig. 1). Agonist binding to the AR causes a conformational change that stimulates a heterotrimeric protein, which acts as a molecular transducer. The heterotrimeric G proteins coupled to ARs (G_s, G_q, or G_i) differ depending on the specific AR activated and can even vary depending on the modification status of a single AR (Fig. 1). The activated heterotrimeric protein dissociates into α– and $\beta\gamma$-components *(2)*, each of which can transduce signals and modulate different second messengers, including activation of adenylyl cyclase (G_s), phospholipase C (G_q), and inhibition of adenylyl cyclase (G_i).

Also integral to the AR signaling cascade is the densensitization and down-regulation of AR signaling. This is accomplished primarily by the G protein-coupled receptor kinases (GRKs), which phosphorylate activated ARs, allowing for the subsequent association of the arrestins. The arrestin association leads to inhibition of classical signaling cascades described above via the endocytic process and activation of newly appreciated signaling cascades, including mitogen-activated protein kinases (MAPKs) *(3)*.

2.1.1. β-AR in Heart Failure

The ARs most predominant in both the cardiac and respiratory setting include β-ARs. The β-AR family consists of three subtypes, β_1, β_2, and β_3. The majority of

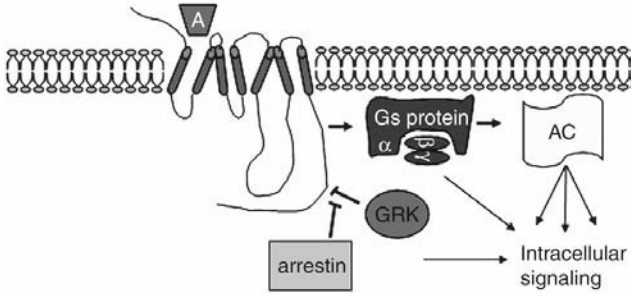

Fig. 1. The β-AR system in cardiomyocytes. On agonist binding to β-ARs, the G_s heterotrimeric protein dissociates into α- and βγ-components. The α-component activates adenylyl cyclase (AC), which results in cAMP accumulation. cAMP activates protein kinase A, which leads to downstream signaling effects, including phosphorylation of L-type calcium channels, phospholamban, troponin I, ryanodine receptors, myosin-binding protein C, and protein phosphatase inhibitor-1 *(4)*. β-ARK1 (or GRK2) is brought to the membrane via association with the G protein βγ-subunits, whereas GRK5 is already associated with the membrane. Either of these GRKs is capable of phosphorylating the agonist-activated β-AR and subsequently desensitizing the receptor. On GRK phosphorylation, a member of the arrestin protein family binds and stimulates an entirely new signaling cascade unique from the adenylyl cyclase. This signaling cascade activates the family of MAPKs.

research to date has primarily focused on the β_1- and β_2-AR subtypes, and the role of the β_3-AR remains controversial *(4)*. The β-AR system is compromised in both the failing heart *(4)* and asthmatic lungs *(5)*. The alterations that take place in the β-AR system during the progression of heart failure are best characterized *(6)*. As the heart begins to fail, compensatory mechanisms are initiated to maintain cardiac output and systemic blood pressure. One of these mechanisms involves the sympathetic nervous system, which increases its myocardial outflow of norepinephrine in an attempt to stimulate contractility *(7)*, leading to β-AR desensitization. There is a reduction of cardiac β-AR density in the failing human heart, and the remaining receptors appear to be desensitized *(8)*. β_1-ARs have been shown to be selectively reduced, and β_2-ARs are not altered *(9,10)*.

Interestingly, the levels of β-adrenergic receptor kinase 1 (β-ARK1, otherwise known as GRK2) are significantly elevated in human heart failure, representing a potential mechanism for loss of β-AR responsiveness seen in this disease *(9)*. The loss of cardiac β_1-ARs is critical because this translates to a larger percentage of β_2-ARs and α_1-ARs. Thus, signaling from these ARs becomes more important in heart failure. Another potential contributing factor to overall decreased β-AR signaling in heart failure is increased levels of $G_{\alpha i}$ *(11)*. These collective β-AR changes are thought to be adaptive to protect the heart against chronic activation *(6,12)*.

2.1.1.1. Compartmentalization of β-ARs

Although at the macroscopic level β_1- and β_2-AR signaling appears similar, evidence suggests that their signaling consequences are not only distinct, but also they are uniquely regulated. There appears to be compartmentalization *(13)*. The β_2-AR subtype is copurified with cardiomyocyte caveolae, whereas the β_1-AR subtype is more evenly distributed *(14)*. In addition, these two subtypes of β-AR possess distinct abilities to activate adenylyl cyclase, resulting in accumulated cyclic adenosine 5′-monophosphate (cAMP) *(4)*. Furthermore, activation of protein kinase A subsequent to cAMP accumulation phosphorylates β_2-AR, which then allows the receptor to switch from coupling with G_s to G_i, whereas β_1-AR does not undergo this same phenomenon *(15)*. The differences between β_1- and β_2-ARs become even more apparent when the studies are conducted in vivo.

2.1.1.2. β_2-ARs in Cardiac Gene Transfer to Normal Hearts

Through several key in vitro and in vivo studies, it appears that genetic enhancement of β_2-AR density has therapeutic potential for cardiovascular and pulmonary disorders. The benefits of cardiac-specific β_2-AR overexpression were first studied in transgenic mice. With more than 200-fold *(16)* cardiac-specific overexpression of β_2-AR using the α-myosin heavy chain promoter, mice demonstrated significantly greater indices of cardiac performance, including enhanced systolic function and myocardial relaxation *(16,17)*. These mice, when compared with their nontransgenic littermate controls, have the phenotype of maximal β-AR myocardial signaling, both biochemically and physiologically *(16)*. Baseline, nonstimulated cardiac function in mice with cardiac-specific overexpression of β_2-AR is equal to or greater than function in control mice with maximum doses of the β-AR agonist isoproterenol. In addition, there is minimal pathology associated with cardiac β_2-AR overexpression up to 1 yr of age, including negligible fibrosis and collagen replacement *(18)*. A similar phenotype was seen in mouse models with more modest (30- to 50-fold) cardiac β_2-AR overexpression *(19,20,21)*. However, too much β_2-AR overexpression (>200-fold) can lead to cardiac toxicity *(21)*. Importantly, moderate overexpression of the β_2-AR in the heart, using hybrid breeding strategies in a mouse model of heart failure, restores ventricular function and reverses cardiac hypertrophy *(20)*. Therefore, this suggests that β_2-AR supplementation is a potential for gene therapy as a means of enhancing ventricular function.

Gene therapy using an adenovirus that expresses the β_2-AR (adeno-β_2-AR) has been used both in vitro in cultured cardiac myocytes and in vivo. In cultured myocytes, adeno-β_2-AR enhanced adrenergic signaling in cells isolated from hearts of adult control rabbits and those with heart failure *(22,23)*. In vivo delivery of the adeno-β_2-AR using open chest intracoronary injection (aortic cross-

clamp) to normal rabbit hearts produced diffuse multichamber myocardial expression with a reproducible 5- to 10-fold β-AR overexpression in the heart, which at 7 and 21 d after delivery resulted in increased in vivo hemodynamic function compared with control rabbits that received an empty adenovirus *(24)*. Several physiological parameters, including contractility, were significantly enhanced basally and showed increased responsiveness to the β-AR agonist isoproterenol *(24)*. Percutaneous left circumflex artery-mediated gene transfer of adeno-β_2-AR to normal rabbit hearts produced expression in a chamber-specific manner, with approx 10-fold overexpression of the β_2-AR *(25)*. Delivery of a control virus that expresses the β-galactosidase gene did not alter in vivo left ventricular systolic function, whereas overexpression of β_2-ARs in the left ventricle improved global left ventricular contractility at baseline and in response to isoproterenol *(25)*. In addition, in a rat model of heterotopic cardiac transplantation, ex vivo delivery of adeno-β_2-AR prior to heterotopic transplantation resulted in enhanced function 1 wk later *(26)*. Therefore, similar to what was seen in transgenic mice, cardiac-specific overexpression of β_2-ARs using adenovirus in either a global or chamber-specific manner or ex vivo in a transplant situation is sufficient to improve baseline and agonist-stimulated cardiac function.

2.1.1.3. β_2-ARs in Cardiac Gene Transfer to Failing Hearts

Adenoviral transfer of the β_2-AR is also capable of improving failing hearts. Pressure overload is a method used in animals to induce cardiac hypertrophy and failure. Concomitant with the failure, there is a decrease in β-AR responsiveness and receptor number *(1)*. In vivo transfection of β_2-AR enhances the cardiac response to isoproterenol in the pressure-overloaded rat heart, thus preserving myocardial function *(27)*. In addition, as a model of cardiac unloading, such as that which occurs with the use of left ventricular assist devices, rabbits undergoing heterotopic transplantation of failing hearts with prior treatment with intracoronary delivery of adeno-β_2-AR functionally recovered rapidly, and this improvement in function was comparable to nonfailing hearts *(28)*. These data suggest that β_2-AR may be a useful molecular adjunct to existing therapies in select patients with heart failure.

Interestingly, because of the dual coupling of β_2-AR, and not β_1-AR, to both G_s and G_i, it appears that β_2-AR–G_i coupling conveys a significant cell survival signal that counteracts apoptosis induced by concurrent $\beta_{1/2}$-AR–G_s-mediated and other signaling pathways *(29)*. This survival pathway sequentially involves G_i, $G_{\beta\gamma}$, phosphoinositide-3 kinase, and Akt *(29)*. This suggests that selective activation of cardiac β_2-ARs may provide beneficial effects to the failing heart via catecholamine-dependent inotropic support without cardiotoxic consequences *(29)*. Further, it suggests that β_2-ARs are excellent targets for gene transfer-based gene therapy in the failing heart.

2.1.1.4. β_2-AR Gene Transfer for Arrhythmias and Heart Rate Control

Arrhythmias and heart rate control are complicating factors associated with heart failure. β-ARs can affect the automaticity of myocardium; accordingly, the use of β_2-AR gene transfer has been explained for this purpose. Studies were done that injected β_2-AR plasmid constructs into the right atrium of native murine hearts *(30)*. Mouse hearts that were transfected with β_2-AR and subsequently heterotopically transplanted had a marked increase in cardiac rate as compared with mice transfected with control plasmids *(30)*. Minimal changes were noted in the electrocardiograms of β_2-AR-transfected hearts, suggesting that electrical conduction is unaltered except for the increased basal heart rate. These studies demonstrated that the basal heart rate can be enhanced by local delivery of β_2-ARs, improving cardiac automaticity and suggesting that the β_2-AR may be a successful candidate gene to act as an in vivo alternative to pacemaker implantation.

2.1.1.5. β_2-AR in Pulmonary Disease

Not only has gene transfer of the β_2-AR been successful in the improvement of heart function, β_2-AR gene therapy has also been successful in the treatment of animal models of asthma and pulmonary edema *(31)*. β-AR agonists accelerate the clearance of edema from the alveolar airspace by increasing the function of epithelial transport proteins. Adeno-β_2-AR was used to cause a significant increase in β_2-AR number and function in the alveolar epithelium of normal rats *(31)*. β_2-AR overexpression upregulates alveolar fluid clearance, improves responsiveness to endogenous catecholamines, and prevents receptor desensitization, suggesting a therapeutic role for the β_2-AR in the treatment of pulmonary edema *(31)*. In lung airway smooth muscle, β_2-ARs also act to relax the muscle, resulting in bronchodilation, and contribute to bronchomotor tone *(32)*. In asthma, there is excessive bronchial smooth muscle contraction, and airway epithelial and smooth muscle β_2-AR function is depressed *(32)*. Although current therapy for the disease includes the regular use of β-agonists for bronchodilation, this therapy also results in β-AR desensitization, thus potentially worsening obstruction and limiting the effectiveness of therapy *(32)*. β_2-AR gene delivery may be a successful strategy to treat asthma because transgenic overexpression of β_2-AR in airway smooth muscle results in mice resistant to an animal model of bronchoconstriction *(33)* and hyperreactivity *(34)*, although it remains to be determined whether this is the case.

2.1.2. β_1-ARs in Heart Disease

Interestingly, although β_2-ARs appear to be beneficial during disease states, it appears that β_1-ARs, the most abundant β-AR subtype in the human heart, are

pathological *(1)*. Even at modest levels of cardiac-specific β_1-AR overexpression in the range of 3- to 15-fold, transgenic mice present with myocardial hypertrophy with rapid progression to failure *(35,36)*. In vitro studies of cardiac myocytes demonstrated that prolonged stimulation of β_1-AR induces cAMP-independent calcium-calmodulin kinase II-dependent apoptosis *(37)*, whereas β_2-AR stimulation may actually prevent apoptosis *(29)*. In addition, it appears that β_1-AR stimulation also leads to cardiac fibrosis and accumulation of extracellular matrix *(38)*. Therefore, at least with respect to cardiac failure, it does not appear that overexpression of the β_1-AR would be a successful approach to improve cardiac function. However, data suggest that the use of antisense therapy against the β_1-AR could be a useful strategy to combat high blood pressure *(39)*, and perhaps this strategy could also be used in the heart to minimize the detrimental β_1-AR effects caused by prolonged stimulation.

2.2. *G Proteins in Heart Failure*

2.2.1. G_i

In addition to enhancing β_2-ARs or inhibiting β_1-ARs, potential gene therapy can also be administered downstream of the AR in the signaling cascade. As determined using mice with G_{i2} gene ablation, G_{i2} is critical for the prevention of hypertrophy and survival of mice with chronic β_2-AR signaling *(40)*. Although it remains unclear whether $G_{\alpha i2}$ upregulation is part of the diminished positive inotropic effect after β-AR stimulation or whether it represents a protective mechanism to attenuate the effect of adrenergic overstimulation, gene transfer of $G_{\alpha i2}$ severely attenuated the β_1-adrenergic contractile response in cardiac myocytes isolated from normal adult female rabbits *(41)*. Therefore, this would suggest that it may be advantageous to increase $G_{\alpha i2}$ levels such that beneficial antiapoptotic G_i-mediated β_2-AR signaling *(29)* is enhanced and detrimental β_1-AR signaling, in the setting of heart failure, is diminished. Another possibility is the use of gene therapy of the $G_{\alpha i2}$ as an antiarrhythmic strategy. $G_{\alpha i2}$ overexpression in the atrioventricular node using adenoviral gene transfer suppressed baseline atrioventricular conduction and slowed the heart rate during atrial fibrillation without producing complete heart block *(42)*. In essence, the $G_{\alpha i2}$ was acting as a directed β-AR antagonist again, inhibiting detrimental β-AR signaling. Therefore, gene therapy for cardiac-specific overexpression of the $G_{\alpha i2}$ warrants further investigation to determine whether it is a successful strategy to augment beneficial and attenuate detrimental β-AR signaling with respect to cardiac contractility and pacing.

2.2.2. G_s

In contrast to $G_{\alpha i}$ signaling, which is enhanced in heart failure, $G_{\alpha s}$ protein levels are unchanged *(43)*. However, studies have been done using transgenic

cardiac overexpression of $G_{\alpha s}$, and it was found that the efficacy of the β-AR–G_s–adenylyl cyclase signaling pathway is enhanced *(44)*. This increased G_s activity leads to amplified inotropic and chronotropic responses to endogenous sympathetic stimulation, which over the life of the animal results in myocardial damage characterized by cellular degeneration, necrosis, fibrosis, and compensatory hypertrophy *(44)*. Therefore, similar to β_1-AR, a gene therapy approach targeting this molecule would not be to enhance but rather to inhibit signaling. This could be accomplished through the use of a peptide inhibitor of G_s signaling *(45)* engineered in a similar manner to that described for G_q signaling in the heart *(46)*, although this remains to be determined.

2.2.3. G_q

Activation of $G_{\alpha q}$ signaling in the heart through either cardiac overexpression of $G_{\alpha q}$ *(20)* or excessive activation of receptors that couple to G_q, including α_1-ARs, can induce cardiomyocyte hypertrophy *(47)*. Thus, inhibition of G_q and its signals was envisioned by us as a potential therapeutic intervention to limit cardiac hypertrophy, which often leads to heart failure in humans. To achieve class-specific G protein inhibition and inhibit the signaling of all receptors that employ G_q, we targeted the receptor–G_q interface *(46)*. This therapeutic strategy eliminates the need for multiple receptor antagonists in a variety of diseases, including pressure overload cardiac hypertrophy. We designed an inhibitor carboxyl-terminal peptide of $G\alpha_q$ that contains the region of the $G_{\alpha q}$ subunit that interacts with the intracellular domains of agonist-occupied receptors (GqI) and created transgenic mice with cardiac-specific overexpression of this GqI peptide *(46)*. When pressure overload was surgically induced, the GqI transgenic mice developed significantly less ventricular hypertrophy than control animals; therefore, inhibition of myocardial G_q may be a possible strategy for preventing pathophysiological signaling by simultaneously blocking multiple receptors coupled to G_q *(46)*. This peptide inhibitor strategy is particularly amenable to targeted gene therapy strategies because it would permit organ-specific inhibition of an entire class of receptors and minimize side effects. In addition to cardiac hypertrophy, this strategy of targeting G_q signaling may be amenable for hypertension because G_q signaling can cause vasoconstriction, which plays a role in this vascular disorder. Further studies will be directed in this area.

2.3. GRKs in Cardiovascular Disease

2.3.1. β-ARK1 (GRK2)

Much work on cardiovascular gene therapy has been done in our lab targeting the manipulation of β-ARK1 (GRK2) activity. Signaling via ARs is regulated by GRKs, and β-ARK1 is upregulated in heart failure *(9)*. Evidence from

transgenic mouse models suggests that inhibiting β-ARK1 may be beneficial in the setting of heart failure. On agonist binding, β-ARK1 is translocated to the membrane via the βγ-subunits of the heterotrimeric G protein *(18)*. Overexpression of the carboxyl terminal portion of β-ARK1 (β-ARKct) competes with endogenous β-ARK1 and prevents the translocation of β-ARK1 and its subsequent phosphorylation and desensitization of its target G protein-coupled receptor *(48)*. There are numerous different G protein-coupled receptors in the heart that β-ARK1 desensitizes *(18)*. In addition, β-ARKct also interferes with βγ-signaling, including activation of the family of MAPKs *(48)*. However, in the heart, the β-ARs are the predominant receptor; therefore, it is believed that the majority of β-ARKct actions are caused by inhibition of β-ARK1 activity rather than effects on other signaling systems, although this remains to be determined *(2)*.

Importantly, mice with transgenic cardiac-specific expression of the β-ARK1 demonstrated attenuation of agonist-stimulated left ventricular contractility in vivo, dampening of myocardial adenylyl cyclase activity, and reduced functional coupling of β-ARs *(48)*, similar to what is observed in heart failure. In contrast, mice expressing the β-ARKct displayed enhanced cardiac contractility in vivo both basally and with agonist stimulation *(48)*, indicating an important role for β-ARK1 in normal cardiac regulation and function *(1)*. In fact, the β-ARKct has been able to restore normal β-AR function and improve left ventricular function and remodeling, cardiac hypertrophy, and survival rates in several different mouse models of heart failure *(1)*. Therefore, these studies were applied to larger animal models of heart failure to determine whether gene therapy using an adeno-β-ARKct would be a successful therapeutic strategy.

Adeno-β-ARKct infection transmitted globally to the entire heart was able to prevent the development of heart failure in a rabbit following left circumflex artery ligation if given at the time of ligation *(49)* or reverse the heart failure phenotype if given 3 wk following myocardial infarction via percutaneous subselective coronary artery catheterization *(50)*. In addition, inhibition of β-ARK1 activation using adeno-β-ARKct was able to restore β-AR signaling and contractile function in donor hearts that had undergone cardioplegic arrest and cold ischemia for up to 4 h prior to transplant. Thus, β-ARK1 inhibition may represent a novel target in limiting depressed ventricular function after cardiopulmonary bypass *(51)*. Interestingly, β-ARK1 levels are also increased in human hypertensive patients *(52)*. Therefore, it would be interesting to determine whether adeno-β-ARKct would be a successful antihypertensive therapeutic strategy. Importantly, we have data supporting this idea as we overexpressed β-ARK1 in the vascular smooth muscle of transgenic mice, and this was sufficient to cause hypertension *(53)*.

2.3.2. GRK5

Unlike human heart failure, for which there has been no change in GRK5 documented, in some animal models of heart failure such as the pacing-induced pig model *(54)*, cardiomyopathic hamsters *(55)*, and rats with surgically induced myocardial infarction *(56)* and hypertension *(57)*, GRK5 levels are increased. GRK5 also phosphorylates and desensitizes β-ARs as well as other ARs. Therefore, it might be of potential therapeutic benefit to inhibit GRK5 using an adenoviral approach to express small molecule interfering RNA (RNAi) inhibitors that could prevent RNA transcription of the GRK5 gene. Alternatively, some sort of peptide inhibitors of GRK5 could be derived that would inhibit GRK5 function and thus potentially restore heart function.

2.4. Regulators of G Protein Signaling Proteins

In addition to the GRKs, a new class of proteins has been appreciated; the regulators of G protein signaling (RGS) proteins also exhibit specific regulation of G protein-coupled receptor-induced signaling within cells *(58)*. RGS proteins negatively regulate the activity of heterotrimeric G proteins by accelerating guanosine 5′-triphosphate hydrolysis and termination of signaling *(58)*. To date, it has been described that the RGS proteins have a relatively nonspecific negative regulation of G protein-coupled receptor signaling mediated by $G_{i/o}$ and $G_{q/11}$, and an interaction with G_s and G_{12} has not been detected *(58)*. The majority of cardiovascular studies to date have focused on RGS4, although there are 13 different RGS proteins expressed in the heart and vasculature *(58)*. Transgenic mice with cardiac-specific overexpression of RGS4 appear normal basally with no apparent morphological abnormalities *(59)*. However, the hearts of RGS4 mice are markedly compromised in their ability to adapt to pressure overload induced by transverse aortic constriction, and they had elevated postoperative mortality compared to nontransgenic littermate control mice *(59)*. In contrast, when RGS4 mice were mated with a heart failure mouse model in which $G_{\alpha q}$ signaling is enhanced, the RGS4 was able to delay the progression of heart failure *(60)*. Therefore, the antihypertrophic effects that RGS4 can exert on $G_{\alpha q}$ signaling in the heart can be either beneficial or detrimental depending on the physiology or pathophysiological context, suggesting that further studies are needed to explore whether the RGS family of proteins may be a potentially important therapeutic target to either enhance or inhibit, depending on circumstances.

2.5. Adenylyl Cyclase Gene Transfer in Heart Disease

β-AR signaling is coupled to adenylyl cyclase. When β-ARs are coupled with G_s, as is primarily the case, β-AR stimulation activates adenylyl cyclase,

resulting in an accumulation of cAMP and activation of protein kinase A, which in the heart lead to increased chronotropy and inotropy. Protein kinase A activity phosphorylates a number of important and interesting substrates, including β_2-AR, such that the β_2-AR is now capable of coupling to G_i, inhibiting adenylyl cyclase activity and preventing apoptosis *(29)*. Adenylyl cyclase 5 and 6 are the most abundantly expressed cyclases in the heart *(61)*. Interestingly, cardiac-specific overexpression of adenylyl cyclase 6 alone had normal cardiac function with no change in myocardial β-AR; G protein or cAMP expression and signaling were only altered when transmembrane receptors were activated *(62,63)*. Cardiac-specific adenylyl cyclase 6 mice were mated with a mouse model of heart failure, and the hybrid mice had increased survival, restored cAMP-generating capacity, improved basal heart function, and increased β-AR responsiveness *(64)*. This suggested that adenylyl cyclase 6 may be a powerful therapeutic target. In fact, intracoronary injection of a recombinant adenovirus encoding adenylyl cyclase 6 into normal pigs provided persistent increases in cardiac function, whereas basal heart rate and blood pressure were unchanged *(65)*. Therefore, although further study is needed, these data suggest that long-term exposure to cardiac-selective overexpression is beneficial and that this may also be an important method for increasing function in the setting of heart failure.

3. Conclusions

Gene therapy is a powerful research and therapeutic tool that allows for organ-specific expression of transgenes. As an investigational tool, gene therapy of the AR system is powerful because it allows for acute changes in expression/activity to be studied without developmental issues and chronic expression that is encountered with transgenic mice. As a therapeutic approach, there are many different molecules along the cascade involved in AR signaling pathways that can be considered as targets, which may provide beneficial outcomes in the setting of heart failure, asthma, hypertension, and other diseases of the cardiovascular and pulmonary systems. Further research is needed to determine whether a single target approach would be more successful than a multimolecular approach. In addition, it remains to be determined whether a designer strategy is needed in which transgenes are manipulated and tailored prior to infection such that certain signaling pathways are favored over others *(32)*. What has been established through extensive transgenic mouse models and larger animal models using adenoviral-mediated gene delivery is that the genetic manipulation of several members of the AR signaling cascade has therapeutic potential that may lead to novel strategies to treat diseases for which, overall, current drug treatments are not optimal.

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