REVIEW
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Alterations of β-Adrenergic Receptor Signaling in Cardiac Hypertrophy and Heart Failure: β-Adrenergic Receptor Desensitization in Cardiac Disease

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ABSTRACT

β-adrenergic receptors (βAR) belong to the large family of G protein-coupled receptors that form the interface between the sympathetic nervous and cardiovascular systems. G protein-coupled receptors undergo adaptation to repeated or prolonged agonist stimulation, which is termed desensitization. Significant βAR desensitization occurs with the development of cardiac hypertrophy and heart failure, and uncoupling of βARs and defects in this pathway might be primary elements underlying the transition from compensated to uncompensated cardiac failure. Decreasing the level of myocardial βARK1 in established heart failure is a novel approach to improving impaired βAR receptor function, and potentially alter the pathogenesis of this disease. **(Korean Circulation J 2005;35:485-492)**

KEY WORDS : Beta-adrenergic receptor kinase ; Cardiac hypertrophy ; Heart failure.

Introduction

The most abundant receptors on the cell surface area of neurotransmitters are members of the large superfamily of G protein-coupled receptors (GPCRs). They share similar primary structures, a common seven-transmembrane-spanning domain architecture, and modulate the intracellular metabolism through the activation of heterotrimeric GTP-binding proteins (G proteins). One major mechanism controlling GPCR responsiveness is the activation-dependent regulation of receptors, also called homologous desensitization.[1] Other mechanisms also contribute to intrinsic regulation of GPCR signaling, including receptor activation-independent regulation of receptors, or heterologous desensitization, as well as mechanisms that act after the receptors themselves, through direct regulation of the G proteins or by altering the signaling efficacy of downstream effectors. The activated state of GPCRs serves not only as activators of G proteins, but also as the substrate for protein phosphorylation by a family of protein kinases, known as GPCR kinases (GRKs). GRKs can discriminate between the inactive and agonist-activated state of a receptor, partly because they are catalytically activated by stimulated receptors. Thus, activated receptor regulation by GRKs results in homologous desensitization (Fig. 1).[2] There are seven known GRK subtypes, which are classified into three subfamilies (GRK1/7, GRK2/3 and GRK4/5/6) based on their sequence and functional similarities. One of these families is visual (GRK1/7), where as one of the others is expressed primary in testes (GRK4). Thus, four GRK subtypes (GRK2, GRK3, GRK5 and GRK6) must account for regulation of most of the GPCRs found throughout the body.[3]

The control of cardiac contractility resides in the ability of the cell to regulate intracellular Ca^{2+}, mediated predominately through stimulation of the β-adrenergic receptor (βAR).[4] βARs belong to the large family of G protein-coupled receptors that form the interface between the sympathetic nervous and cardiovascular systems.[5] Binding of β-agonists to the βAR leads to activation of adenylyl cyclase, generating cAMP and activating cAMP-dependent protein kinase A (PKA). Phosphorylation of critical regulatory proteins by PKA act in concert to enhance transsarcolemmal Ca^{2+} influx, decrease sensitivity of the contractile system to Ca^{2+}, and most importantly, increase Ca^{2+} uptake into the sarcoplasmic reticulum (SR). The overall physiological effect following βAR stimulation is to increase the rates[1][2] of contrac-

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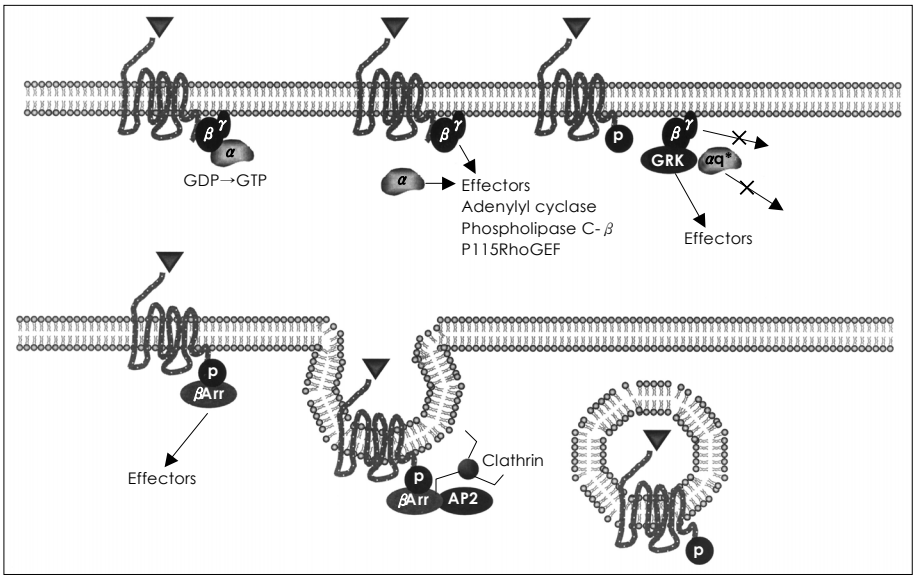

Fig. 1. Schematic diagram representing the key steps in GPCR signaling and homologous desensitization. See text for details. GPCR: G protein-coupled receptor, GDP: guanosine diphosphate, GTP: guanosine triphosphate, GRK: G protein-coupled receptors kinase, βArr: β-Arrestin, AP2: adaptor protein 2.

tion, peak force[3] and relaxation.[6] This review focuses on the normal regulation of the βAR system and the pathological conditions of cardiac hypertrophy heart failure.

β AR Regulation and Desensitization

The βAR belongs to the G protein-coupled receptor superfamily, which is characterized by a seven hydrophobic transmembrane region, with an extracellular N terminus and a cytoplasmic C terminus.[7][8] Domains critical for interaction with G proteins have been localized to the second and third cytoplasmic loops and the C terminus. Three distinct human genes encoding the βAR subtypes β1, β2 and β3 have been identified.[9][10]

G protein-coupled receptors undergo adaptation to repeated or prolonged agonist stimulation, which is termed desensitization. Regulatory mechanisms that contribute to β AR desensitization can be broadly classified into those that affect the receptor function (uncoupling) and those that affect the receptor number (downregulation).[11-13] Downregulation of receptors in response to prolonged exposure to agonist is a slow process (occurring over hours) and contributes to the longer-term desensitization of receptor function. Mechanisms mediating this process include changes in the rate of receptor turnover, receptor gene transcription and mRNA turnover.[14][15]

In contrast to receptor downregulation, agonist-induced loss of receptor function can occur within minutes of receptor stimulation and are mediated by uncoupling of the receptors from G proteins. β AR uncoupling occurs through the rapid phosphorylation of the receptor by two types of kinases: second messenger kinases and the G protein-coupled receptor kinases (GRKs).[14-17] Second messenger kinases, cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) have been shown to phosphorylate the βAR within the third cytoplasmic loop, which contains a consensus sequence (RRSS) for PKA phosphorylation.[8] Since PKA and PKC phosphorylate multiple proteins, they mediate a generalized cellular hypo-responsiveness, known as heterologous desensitization.[12][18] PKA mediated phosphorylation of the β_2AR also serves to switch coupling from G_s to G_I.[19] Recent evidence has shown that phosphorylation of the β_2AR by PKA not only result in a decreased receptor-coupling efficiency to G_s, but leads to GBY-subunit dependent mitogen-activated protein (MAP) kinase activation by enhancing β_2AR coupling to G_I.[19]

One of the most important mechanisms for rapidly regulating the β_1 and β_2AR functions is the agonist-stimulated receptor phosphorylation by GRKS, resulting in decreased sensitivity to further catecholamine stimulation.[11][12] GRKs phosphorylate only agonist-occupied receptors, leading to a process known as homologous desensitization.[12][13] The β-adrenergic receptor kinase (βARK1) is a member of a family of at least 7 GRKs, which phosphorylate and regulate a wide variety of receptors and couple to heterotrimeric G proteins.[10][16][18] When β ARs are activated by agonist heterotrimeric G proteins they dissociate into Gα and $G_{\beta\gamma}$ subunits. The $G_{\beta\gamma}$ subunit complex is membrane anchored by a lipid group (geranylgeranyl), and is able to target βARK1 to the membrane through direct physical interaction; thus, facilitating phosphorylation of activated receptors.[20][21] As with the β_2AR, the phosphorylated receptors bind

to inhibitory proteins, the β-arrestins, which uncouple the receptor from G_s.[22] A key role for β-arrestins in receptor internalization has recently been appreciated. Recent evidence suggests that β-arrestins function as clathrin adaptors, which target the ligand-occupied receptors into clathrin-coated pits for internalization.[23] Following receptor endocytosis into endosomal vesicles, receptors are either recycled back to the plasma membrane or targeted for degradation.[12][24]

It has also been recognized that the $G_{\beta\gamma}$ dimer plays an important and diverse regulatory role in transmembrane signaling in the heart.[25] For example, $G_{\beta\gamma}$ activation of the Ik,ach channel in atrial tissue following A_1-adenosine or M_2-muscarinic receptor stimulation underlies the vagal slowing of the heart rate.[26] Signaling through G_i-coupled receptors mediate signals that are carried by $G_{\beta\gamma}$ dimers, which can lead to activation of MAP kinase through a Ras-dependent pathway.[27] Since different combinations of $G_{\beta\gamma}$ isoforms have preferential affinity for βARK1, this may provide a mechanism for specificity in GRK-receptor-interactions.[28]

βAR Desensitization in Cardiac Disease

Cardiac hypertrophy

Cardiac hypertrophy represents one of the most important adaptive responses to increased mechanical load on the heart. In an attempt to normalize excessive forces and work performed per contractile unit, myocardial hypertrophy unloads the heart by adding new sarcomeres in order to distribute tension across a greater cellular mass.[29] While the induction of cardiac hypertrophy can be viewed as a corrective response to elevated stress, it is now clear that sustained hypertrophy initiates a myopathic process, which leads to uncompensated heart failure.[30][31] The molecular mechanisms responsible for this transition, from a compensatory state to one of progressive chamber enlargement and myocardial failure, are not well understood. Derangement in a number of cellular processes have been implicated in this pathological transition, including abnormalities in βAR signaling,[32] activation of MAP kinase pathways[33] and impaired ability of the L-type Ca^{2+} channel to activate Ca^{2+}-induced Ca^{2+} release.[34] Recent evidence suggests that signals transduced through G protein-coupled receptors are critical in the initiation of the hypertrophic program.[35] These receptor-coupled signals may participate in the progressive deterioration of cardiac function that occurs with chronic stress.[36] In this regard, an obligatory role for the heterotrimeric guanine nucleotide binding protein, Gq, in the initiation of ventricular hypertrophy *in vivo* was recently shown using a transgenic mouse model overexpressing an inhibitor of Gq coupling.[37]

In some experimental models of cardiac hypertrophy, a number of abnormalities in βAR signaling have been found, including depressed isoproterenol stimulated adenylyl cyclase activity and elevated levels of $G_{1\alpha}$.[38] To determine the molecular mechanisms for βAR desensitization in cardiac hypertrophy, we performed experiments in transgenic mice with cardiac hypertrophy created through transverse aortic constriction (TAC).[39][40] Pressure overload hypertrophy, induced by TAC in wild-type mice, resulted in marked βAR desensitization, which was associated with a threefold increase in βARK1 activity[41] (Fig. 2A, C). To demonstrate that βAR desensitization in cardiac hypertrophy was secondary to the augmented βARK1 levels, cardiac hypertrophy was induced in transgenic mice overexpressing a βARK1 inhibitor (βARKct), which has previously been shown to block βAR desensitization and have enhanced contractility.[42] In βARK1 inhibitor transgenic mice exposed

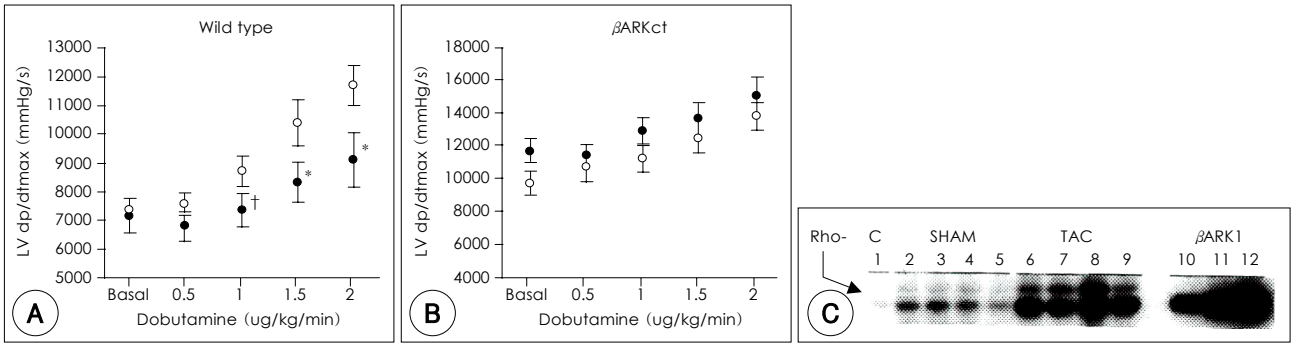

Fig. 2. In vivo contractile function in wild-type and transgenic mice overexpressing a βARK1 inhibitor (βA-RK1ct) following the development of cardiac hypertrophy induced through transverse aortic constriction (TAC). Cardiac catheterization was performed in the intact anesthetized mice using 1.8 Fr. High-fidelity micromanometer, before and after progressive infusion of dobutamine, in sham (○) and TAC (●) wild-type mice (A). $P<0.05$ sham vs. TAC. No significant difference was seen between the sham (○) and TAC (●) βARK1 inhibitor mice (B). Cytosolic extractions from sham-operated and TAC wild-type hearts were measured for their capacity to phosphorylate the G protein-coupled receptor, rhodopsin. Phosphorylated rhodopsin was visualized by autoradiography following electrophoresis through a 12% SDS-polyacrylamide gel. The cytosolic extraction from TAC hearts had an approximate threefold increase in GRK activity compared to that from hearts of sham-operated animals (Adapted with permission from ref.40). βARK1: β-adrenergic receptor kinase, βARK1ct: β2-adrenergic receptor kinase c-terminal.

to the same level of pressure overload, βAR desensitization was completely reversed, despite the development of significant cardiac hypertrophy[41] (Fig. 2B). Thus, significant βAR desensitization occurs with the development of pressure overload cardiac hypertrophy, and the uncoupling of βARs in cardiac hypertrophy can be accounted for by an increase in βARK1.

In patients with hypertension, cardiac hypertrophy develops as an adaptive process, which allows the heart to normalize after load and preserve systolic performance. However, it has been established that the echocardiographically determined increased left ventricular mass is an important independent predictor of cardiovascular morbidity and mortality in patients with hypertension. Although increased blood pressure is considered an important factor promoting cardiac hypertrophy, only a portion of the observed variance in the left ventricular mass is accounted for by blood pressure. Other clinical and neurohumoral factors, including the β-adrenergic system, have been implicated as contributors. There is increasing evidence of a considerable contribution to the activation of the sympathetic nervous system in cardiac hypertrophy. Catecholamines are susceptible to local blood flow and tissue-specific spillover, which limit their ability to accurately reflect the magnitude of sympathetic activation, and they provide little information on the sympathetic traffic in specific tissues. Hypertensive individuals with cardiac hypertrophy have greater sympathetic activation than those without, as indicated by an increase in unitary firing frequency and fiber recruitment. In addition, a recent study demonstrated that cardiac noradrenaline spillover was greater in hypertensive patients with cardiac hypertrophy than in those without, whereas the total systemic noradrenaline spillover was no different. This finding may indicate that greater sympathetic activation, reflected by greater expression of βARK1, can contribute to the development of cardiac hypertrophy in hypertension. The lymphocyte expression of βARK1 and GRK activities were enhanced to a significantly greater extent in hypertensive patients with cardiac hypertrophy than in those without (Fig. 3).[43] These findings suggest that more potent sympathetic stimulation, reflected by the expression of βARK1, may be required for the development of cardiac hypertrophy in hypertension.

Chronic heart failure

Activation of the sympathetic nervous system is considered one of the cardinal pathophysiologic abnormalities in patients with heart failure,[44-46] which frequently precedes the development of overt symptoms.[47] Plasma norepinephrine and renin activities are increased in patients with heart failure, and are known prognostic factors for survival.[45][48] Elevated circulating norepinephrine and epinephrine have been implicated in contributing to the profound βAR downregulation and receptor uncoupling characteristic of end-stage human dilated cardiomyopathy,[49-51] a process mediated, in part, by βARK1.[13] In a variety of human and experimental conditions, βAR desensitization, in response to catecholamine stimulation, has been shown to be associated with heightened levels of βARK1.[41][52][54] In chronic human heart failure, reduced agonist-stimulated adenylyl cyclase activity, due to both a diminished receptor number and impaired receptor function, is a consistent feature.[55] In end-stage human heart failure, these changes in the βAR function are associated with elevated mRNA levels and activity for βARK1.[52][56] Results from transgenic mice overexpressing βARK1 and GRK5[42][57] demonstrate how the up-regulation of these molecules in a diseased heart could markedly alter the βAR function by enhancing receptor desensitization. Furthermore, chronic treatment with either the βAR antagonist bisoprolol in the pig[58] or carvedilol in the mouse[59] (potent therapeutic agent in human heart failure,[60][61] substantially decreases the level of βARK1 activity.

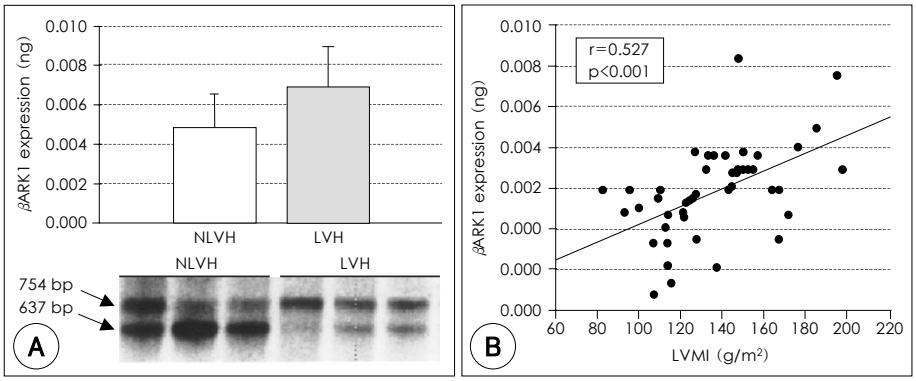

Fig. 3. A: representative competitive reverse transcriptase-polymerase chain reaction from the RNA of lymphocytes obtained from the patients. The level of βARK1 (754 bp) expression was estimated by comparison with an internal control (637 bp), and is presented as the amount (ng) of mRNA expressed per g of total RNA. B: correlation between the left ventricular mass index (LVMI) (n 1/4 49) and the expression of βARK1 in lymphocytes. Correlation coefficients are presented (Adapted with permission from ref. 42). LVH: left ventricular hypertrophy, βARK1: β-adrenergic receptor kinase, LVMI: left ventricular mass index.

To test the hypothesis that the level of cardiac βAR1 activity regulates the myocardial contractile function *in vivo*, a strategy using mouse genetics to create varying levels of βAR1 activity in the heart, coupled with a physiological assessment of contractile function, was performed.[62] Two types of genetically altered mice were used. One group was heterozygous for the targeted disruption of the βAR1 gene, $\beta ARK1^{+/-}$[63]; the second was offspring generated by cross-breeding the previously mentioned transgenic mice containing a βARK1 inhibitor (βARKct) targeted to the heart with the $\beta ARK1^{+/-}$, yielding the double gene targeted line $\beta ARK1^{+/-}$ βARKct.[62] In contrast to the embryonic lethal phenotype of the homozygous βARK1 knockout,[63] the $\beta ARK1^{+/-}$ mice developed normally. Cardiac catheterization was performed in both groups of mice, which showed a stepwise increase in the contractile function in the $\beta ARK1^{+/-}$ and $\beta ARK1^{+/-}$/βARKct mice, with the greatest level observed in the $\beta ARK1^{+/-}$/βARKct animals.[62] The contractile parameters measured in adult myocytes isolated from both groups of gene targeted animals showed a significantly greater increase in the percentage and rate of cell shortening following isoproterenol stimulation in the $\beta ARK1^{+/-}$ and $\beta ARK1^{+/-}$/βARKct myocytes compared to the wild-type cell.[62] Thus, the level of contractile function in the *in vivo* heart can be modulated by the level of βARK1 expression. Furthermore, this has important implications in disease states, such as heart failure (in which βARK1 activity is increased), since even partial inhibition of βARK1 activity will lead to improved functional catecholamine responsiveness.

To address the role of βARK1 and abnormalities of βAR signaling in the pathogenesis of heart failure, we performed experiments in gene-targeted mice with an altered βAR function.[64] Transgenic mice with cardiac restricted overexpression of either the peptide inhibitor of βARK1 (βARKct)[42] or the β_2AR[65] were mated into a genetic model of murine heart failure ($MLP^{-/-}$), closely resembling the phenotype of human dilated cardiomyopathy. This model of dilated cardiomyopathy was created through targeted disruption of the muscle LIM protein (MLP) gene. The MLP is a conserved positive regulator of myogenic differentiation, which may act as a molecular adapter for the promotion of protein assembly along the actin-based cytoskeleton. Hearts from $MLP^{-/-}$ mice are characterized by marked disruption of the cardiomyocyte architecture, depressed cardiac function and markedly abnormal βAR signaling.[66]

The *in vivo* cardiac function of the gene-targeted mice was assessed by noninvasive echocardiography and invasive cardiac catheterization.[64] Both $MLP^{-/-}$ and $MLP^{-/-}$/β2AR mice had enlarged LV chambers, with significantly reduced fractional shortening and a mean velocity of circumferential fiber shortening, demonstrating evidence of profound heart failure (Fig. 4).[64] In contrast, $MLP^{-/-}$/βARKct mice had a normal LV chamber size and preserved cardiac function (Fig 4). The basal LV contractility in the $MLP^{-/-}$/βARKct mice, as measured by LV dP/dtmax, was significantly increased compared to that in the $MLP^{-/-}$ mice; whereas, heightened βAR desensitization was observed in the $MLP^{-/-}$ mice, as measured both *in vivo* (responsiveness to isoproterenol) and in vitro (isoproterenol stimulated membrane adenylyl cyclase activity), overexpression of the βARK1 inhibitor completely restored normal βAR function. An important difference in the two transgenic models is worth noting; whereas, β_2AR overexpression in this model leads to constant maximal signaling,[65] βARKct overexpression preserves normal myocardial βAR responsiveness to endogenous catecholamine stimulation,[42] which may account for the observed difference in outcomes between the $MLP^{-/-}$/β_2AR and $MLP^{-/-}$/βARKct mice. The contrasting phenotypes with observed overexpressions of β_2AR and βARKct show that continuous enhancement of βAR signaling, due to increases in the receptor number, is not sufficient to prevent deterioration of the cardiac function. However, reversal of β_1AR desensitization through the overexpression of the βARK1 inhibitor acts to restore normal G protein coupling of the endogenous uncoupled β_1AR. Interestingly, the

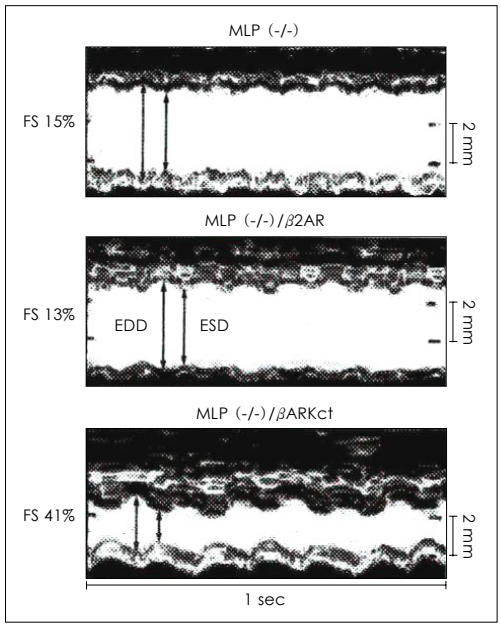

Fig. 4. Echocardiographic analysis of the cardiac function in gene-targeted mice with altered βAR function. Transthoracic M-mode echocardiographic tracings in $MLP^{-/-}$ (upper panel), $MLP^{-/-}$β2AR (middle panel) and $MLP^{-/-}$/βARKct mice (lower panel). The left ventricular dimensions are indicated by the double sided arrow. Both the $MLP^{-/-}$ and $MLP^{-/-}$β2AR mice have chamber dilatation, with reduced wall motion, indicating a depressed cardiac function, whereas the chamber size and cardiac function are normal in the $MLP^{-/-}$ βARKct mouse (Adapted with permission from ref. 60). EDD: end diastolic dimension, ESD: end systolic dimension, MLP: muscle limb protein, β2AR: β2-adrenergic receptor, βARKct: β-adrenergic receptor kinase c-terminal.

deleterious effect of chronic βAR stimulation in the MLP$^{-/-}$/ β_2AR mice was consistent with the experience from clinical studies using oral inotropic agents in severe heart failure.[67]

As mentioned previously, desensitization of agonist-occupied receptors by cytosolic βARK1 requires a membrane-targeting event prior to activation of the enzyme, which is mediated by a direct physical interaction between residues within the carboxyl terminus of βARK1 and the dissociated membrane-anchored $G_{\beta\gamma}$ subunits.[20][21] Since the βARKct peptide used to inhibitor β ARK1 activity *in vivo* functions by sequestering $G_{\beta\gamma}$, it is possible that inhibition of other $G_{\beta\gamma}$-dependent pathways contributes to the benefit observed in the MLP$^{-/-}$/ βARKct animals However, several lines of evidence support the inhibition of βARK1 activity as being the most important mechanism. First, the MLP$^{-/-}$/ βARKct mice have increased responsiveness to isoproterenol, suggesting the inhibition of desensitization. Second, the overexpression of βARKct does not prevent the development of cardiac hypertrophy in response to pressure overload, but reverses βAR desensitization,[41] suggesting that other signaling pathways, critical for the hypertrophic phenotype *in vivo* (such as MAP kinase), are unaffected by the βARKct. Third, the βARK1$^{-/-}$ and βARK1$^{-/-}$/ βARKct mice showed graded increases in sensitivity to βAR agonists, which are associated with a stepwise decrement in the $G_{\beta\gamma}$ dependent βARK1 activity.[62] Finally, other known actions of $G_{\beta\gamma}$,[25][68] such as activation of the Ik, ach channel, adenylyl cyclase and PLC β1-3, appear to be irrelevant in this situation. The $I_{k,ach}$ channel is located in atrial, but not ventricular tissue, and would not be expected to directly alter the contractility.[25][26] The isoforms of adenylyl cyclase regulated by $G_{\beta\gamma}$(I, II, IV) are not found in the heart.[68] PLC β is not activated by myocardial βAR's.[25] Taken together, these data show that restoring normal control of β_1AR signaling by inhibiting desensitization is an important mechanism in the prevention of the progressive deterioration of cardiac function. Furthermore, these studies indicate the potential for a therapeutic strategy aimed to modulate the activity level of myocardial βARK1 in disease states.

Potential novel targets for βARK1 inhibition

New roles for GRK's have recently been appreciated in receptor signaling and desensitization.[11][69][70] In particular, receptor endocytosis and mitogenic signaling,[71] and new nonreceptor substrates for βARK1, such as tubulin,[72] demonstrate how GRKs play a direct role, as agonist-activated kinases, in intracellular signaling.[11] Thus, potential therapeutic applications of a βARK1 inhibitor may, as yet, be unrealized as these putative signaling cascades remain to be identified.

Conclusion

Based on our recent data,[41][43][62][64] and the association that profound alterations in βAR signaling are found in chronic end-stage human heart failure,[73] it is possible that defects in this pathway are primary elements underlying the transition from compensated to uncompensated cardiac failure. Decreasing the level of myocardial βARK1 in established heart failure is a novel approach to improving impaired βAR receptor function and potentially alter the pathogenesis of this disease.

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