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Beta-adrenoceptor signaling pathways mediate cardiac pathological remodeling

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1. ABSTRACT

Beta-adrenoceptors (ARs), members of the G protein-coupled receptor (GPCR) superfamily, play a key role in the rapid regulation of myocardial function. Meanwhile, chronic catecholamine stimulation of adrenoceptors has been proved to be involved in the adverse myocardial remodeling, including cardiac hypertrophy, fibrosis, and apoptosis, which finally develop into heart failure. In the clinical situation, sympathetic hyperactivity is a key factor in the development of heart failure, and beta-blockers greatly improve the outcome of the disease. However, heart failure is still one of the leading causes of death. Therefore, a full understanding of the mechanism of beta-AR-mediated cardiac remodeling could indicate more targets for treating heart failure. This review summarizes a number of important signaling pathways involved in the process of cardiac pathological remodeling under chronic adrenergic stimulation.

2. INTRODUCTION

As members of the GPCR superfamily, beta-ARs exert crucial regulatory functions in both physiological and pathological conditions. The heart expresses three types of beta-ARs: $beta_1$-AR, $beta_2$-AR, and $beta_3$-AR (1). The $beta_1$-AR is the predominant receptor, with a proportion of 60%-80% in the normal myocardium, while the proportion of $beta_2$-AR is 20%-40% (2, 3), and the expression level of $beta_3$-AR being the lowest (4).

Both $beta_1$- and $beta_2$-ARs couple to G_s proteins, which in turn activate adenylyl cyclase (AC) and induce cAMP accumulation and protein kinase A (PKA) activation. PKA phosphorylates various substrates, including the L-type calcium channels (LCCs), phospholamban, ryanodine receptors (RyRs), troponin I and C-proteins, resulting in positively chronotropic, inotropic, and lusitropic effects on the heart (5). Activated

beta$_2$-AR can also couple to G_i, thereby activating the phosphatidylinositol 3-kinase (PI3K) pathway and negating concurrent beta$_2$-AR/G_s-mediated PKA signals. Beta2-AR/Gi/PI3K signaling plays a key role in protecting cardiomyocytes against apoptosis (6-9). Moreover, the functional roles of beta$_2$-AR may vary among different species. In ventricular myocytes from nonfailing rat hearts, the beta$_2$-AR–G_s/cAMP pathway was found leading to relaxation only in the presence of pertussis toxin (10). This suggested that the rat cardiomyocyte beta$_2$-ARs couple more tightly to G_i than to G_s. In contrast, beta$_2$-AR–mediated relaxation could occur *via* the G_s/cAMP pathway in failing human ventricle, implying that the coupling between human cardiac beta$_2$-ARs and G_s is tighter than in the case of G_i (11). Beta$_3$-ARs also can couple to both G_s and G_i proteins (4), but the functional roles of beta$_3$-AR in the heart, especially in the human heart are still not fully understood.

Beta-AR signaling pathways have been shown to be crucial during the cardiac remodeling and dysfunction process (12). Cardiac remodeling is generally accepted as a determinant of the clinical course of heart failure (13). Thus, a full understanding of the mechanism underlying this process would help to find a possible means of reversing pathological remodeling and mitigating cardiac dysfunction. Cardiac remodeling is defined as changes in size, shape, and function of the heart after cardiac injury. Cellular changes including myocyte hypertrophy, fibrosis, apoptosis etc (13). The anatomic and functional remodeling then alters cardiac electrophysiology which terms as electrical remodeling (14). Beta-AR activation is a well-known pro-arrhythmogenic event through increasing sarcoplasmic reticulum (SR) Ca^{2+} load and the frequency of spontaneous SR Ca^{2+} release (15). Recently, Workman AJ (16) summarized the understanding of the involvement of the adrenergic system and its control in atrial fibrillation. In the present review, we will not include the portion concerning electrical remodeling. Here we will focus on the recent work about signaling pathways involved in cardiac hypertrophy, fibrosis, and apoptosis induced by chronic beta-AR activation.

3. BETA-ADRENOCEPTORS AND CARDIAC HYPERTROPHY

Pathological left ventricular hypertrophy (LVH) is a crucial condition triggering several serious cardiac events, including arrhythmias, heart failure, and sudden death (17, 18). Clinical trials suggested that sympathetic hyperactivity might be the key factor of LVH; therefore administration of beta-AR blockers such as metoprolol might alleviate this remodeling process (19, 20). In addition, experiences demonstrated that chronic stimulation of beta-AR with isoproterenol (ISO, non-selective beta-AR agonist) or dobutamine (beta$_1$-selective agonist) could induce cardiac hypertrophy *in vivo* (21, 22). In cultured neonatal rat cardiomyocytes, only stimulation of beta$_1$-AR could lead to cardiac hypertrophy (23). However, transgenic mice overexpressing beta$_1$-AR and those overexpressing beta$_2$-AR eventually develop cardiac hypertrophy (24-26).

The mechanism of cardiac hypertrophy induced by chronic beta-AR stimulation is not fully understood. In the following section, we review a series of key signalling pathways involved in the process of cardiac hypertrophy during chronic adrenergic stimulation.

3.1. G_s/AC/cAMP/PKA signaling

Experiments showed that the classic beta-AR signaling pathway, G_s/AC/cAMP/PKA signaling cascades, not only contributes to regulation of normal heart function, but is also involved in harmful cardiac remodeling. A series of transgenic mice suggested that beta$_1$-AR/G_s/PKA signaling mediate cardiac hypertrophy of chronic adrenergic stimulation. Overexpression of beta$_1$-AR in the heart of transgenic mice initially increases contractile function and responsiveness to ISO, but eventually leads to processive deterioration of cardiac function and cardiac hypertrophy (24). Overexpression of G_s in transgenic animals also induced cardiac hypertrophy (27). Similar findings were obtained with transgenic mice overexpressing PKA (28).

PKA can phosphorylate cAMP response binding protein (CREB)/cAMP response element modulator (CREM), which is a transcription factor, leading to CRE-mediated transcription. CREM inactivation was found to prevent cardiomyocyte hypertrophy, fibrosis, and left ventricular dysfunction in beta$_1$-AR-overexpressing mice (29). The underlying mechanism might involve altered expression of cardiac proteins, including the cardiac RyRs as well as the contractile proteins tropomyosin-1alpha and cardiac alpha-actin (29). However, some studies showed that CREB activation had cardiac protective effects. In spontaneously hypertensive rats (SHRs) which developed cardiac hypertrophy and subsequent heart failure, CREB activation was found to be significantly decreased. Exercise could protect SHRs from cardiac remodeling, with restoration of CREB function in the heart. It may be attributed to the inhibitory effect of CERB activation on cardiac apoptosis by enhancing bcl-2, PGC-1, and mitochondrial gene expression (30).

Inducible cAMP early repressor (ICER), a member of the CREB and CREM family of transcription factors, has a DNA-binding domain identical to that in CREM but lacks the transactivation domain. Therefore it serves as an endogenous inhibitor for CREM/CREB-mediated transcription (31). ICER expression is rapidly induced by beta-AR stimulation in cardiomyocytes, and overexpression of that factor significantly inhibits beta-AR-induced cardiac hypertrophy, while antisense inhibition of endogenous ICER enhances beta-AR-induced increases in protein/DNA content and atrial natriuretic factor (ANF) transcription, which suggests that the degree of hypertrophy is increased (32, 33).

3.2. MAPK signaling cascades

The mitogen-activated protein kinase (MAPK) family is a critical group of factors in intracellular signal transduction and regulation. Classic MAPKs can be divided into three major subfamilies, namely extracellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNK), and p38 MAP kinase. It has been demonstrated that stimulation of beta-AR could activate the three subfamilies

both *in vitro* and *in vivo* (34-36). However, beta-AR-mediated activation of MAPK cascade is complicated and has not been fully understood. In response to beta-AR stimulation, PKA mediated the activation of ERK1/2 and p38 MAP kinase (37-39), and reactive oxygen species (ROS) mediated the activation of JNK (40) in cultured cardiomyocytes. Zhang *et al.* (36) also demonstrated that ROS is an important activator of cardiac MAPK cascades in response to ISO infusion *in vivo*. Recent studies showed that $beta_1$-AR could active ERK through beta-arrestin-mediated transactivation of epidermal growth factor receptor (EGFR), protecting the heart in the face of chronic catecholamine stimulation (41). Those experiments showed multiple signaling of $beta_1$-AR, and it might be possible to explore new receptor blockers, which have the same ability to block the deleterious effects of $beta_1$-AR overstimulation as standard beta-blockers, while simultaneously being able to activate cell protective pathways such as beta-arrestin signaling. We also found a novel PKA-independent, beta-arrestin-1-dependent signaling pathway for p38 activation by $beta_2$-ARs (42).

The importance of MAPK cascades for the regulation of cardiac hypertrophy was proposed based on findings with transgenic mouse model. Overexpression of MAP kinase kinase 1 (MEK1) in the transgenic mouse heart, which showed specific activation of ERK1/2, resulted in considerable cardiac hypertrophy with an increased contractile function (43). The mechanism by which the MEK1-ERK1/2 pathway induces cardiac hypertrophy *in vivo* might be partly attributed to enhancement of the transcriptional activity of nuclear factor of activated T-cells (NFAT), which indicated its crosstalk with the calcineurin-NFAT circuit (44). However, p38 kinase and JNK might have a negative regulatory effect on cardiac hypertrophy. Overexpression of activated MAP kinase kinase 3 (MKK3) or MAP kinase kinase 6 (MKK6) in transgenic mice showing specific activation of p38, induced heart failure in juveniles, which was characterized by abnormal cardiac function, fibrosis, and thinned ventricular walls (45). To the contrary, overexpression of dominant-negative p38alpha induced significant cardiac hypertrophy with normal cardiac function, and rescues cardiomyopathy induced by overexpressed $beta_2$-AR, but not $beta_1$-AR (46). It suggests that p38alpha MAPK mediates the development of cardiomyopathy following chronic $beta_2$-AR stimulation, not $beta_1$-AR stimulation. Similarly, transgenic mice expressing activated MAP kinase kinase 7 (MKK7) in the heart showed specific JNK activation and lethal cardiomyopathy in juveniles without hypertrophy (47). Transgenic mice expressing dominant negative JNK1/2 developed cardiac hypertrophy when subjected to stress stimulation (48). The mechanisms of p38 and JNK inhibiting cardiac hypertrophy remain to be determined, probably contributed to phosphorylating NFAT and reducing the effects of calcineurin signaling in the heart (48, 49). Since different MAPK cascades induced by beta-AR stimulation play different roles in the regulation of cardiac hypertrophy, the integrated effect of these signaling pathways is yet to be thoroughly investigated.

3.3. Ca^{2+}/calcineurin/NFAT signaling

Calcium is an important intracellular signaling factor; an increase in its intracellular concentration is involved in a series of signal transduction pathways regulating cell growth, apoptosis, and excitation-contraction coupling (E-C coupling). The beta-AR induced increase of intracellular Ca^{2+} is a complicated process that has not been fully understood. Influx of extracellular Ca^{2+} and SR Ca^{2+} release are the main sources of intracellular Ca^{2+}, which is at a concentration much lower than the extracellular concentration. Beta-AR mediates PKA activation and subsequent phosphorylation of LCCs and RyRs, which induce rapid increase of intracellular Ca^{2+} (5).

In normal conditions, SR Ca^{2+}-pump can re-uptake Ca^{2+} rapidly, hence the concentration of intracellular Ca^{2+} would not remain at a high level, thereby ensuring a transient increase of intracellular Ca^{2+}, which is essential for excitation-contraction coupling in cardiomyocytes. Under chronic beta-AR stimulation, the inflow of Ca^{2+} is greater than its outflow, and the consequent increase of intracellular Ca^{2+} activates many downstream signaling factors including calcineurin and calmodulin-dependent protein kinase II (CaMKII) (Figure 1). Previous studies observed that calcineurin and CaMKII activities are increased in many animal models of cardiac hypertrophy and heart failure induced by chronic beta-AR stimulation (50-53).

Calcineurin is a serine-threonine phosphatase expressed in multiple tissues including the heart. The physiological role of calcineurin is highly related to its downstream signaling factor NFAT. Calcineurin dephosphorylates NFAT, resulting in translocation of NFAT proteins to the nucleus and activation of many hypertrophic genes (54) (Figure 1). There is evidence supporting the role of calcineurin as a central pro-hypertrophic signaling molecule in the myocardium. Constitutive activation of cardiac calcineurin in a transgenic mouse resulted in massive cardiac hypertrophy and eventually heart failure (55). Conversely, lack of calcineurin A beta in a transgenic mouse model led to a 12% reduction in basal heart size and considerable resistance to various hypertrophic stimuli such as pressure overload, and infusion of angiotensin II (Ang II) and ISO (56). Furthermore, some studies also observed that calcineurin has a critical regulatory effect on cardiac hypertrophy using several endogenous calcineurin inhibitors such as Cabin/Cain. Overexpression of the Cabin/Cain attenuated cardiac hypertrophy induced by pressure overload or ISO (57).

3.4. PI3K signaling cascades

PI3Ks have been linked to some important physiological functions including control of cell growth, survival, and proliferation. The enzymes of PI3K family of can be activated by several receptor tyrosine kinases, such as insulin-like growth factor 1 (IGF-1) (58, 59). Many experiments also found that GPCRs could activate PI3K (6-9, 60). In both neonatal rat cardiomyocytes and adult mouse cardiomyocytes, only $beta_2$-AR stimulation markedly increased PI3K activity and Akt/PKB (AKT) phosphorylation in a G_i-$G_{beta\ gamma}$-dependent manner (6-9, 60) (Figure 1). It is known that with the increase of sympathetic tone, chronic agonist exposure leads to a

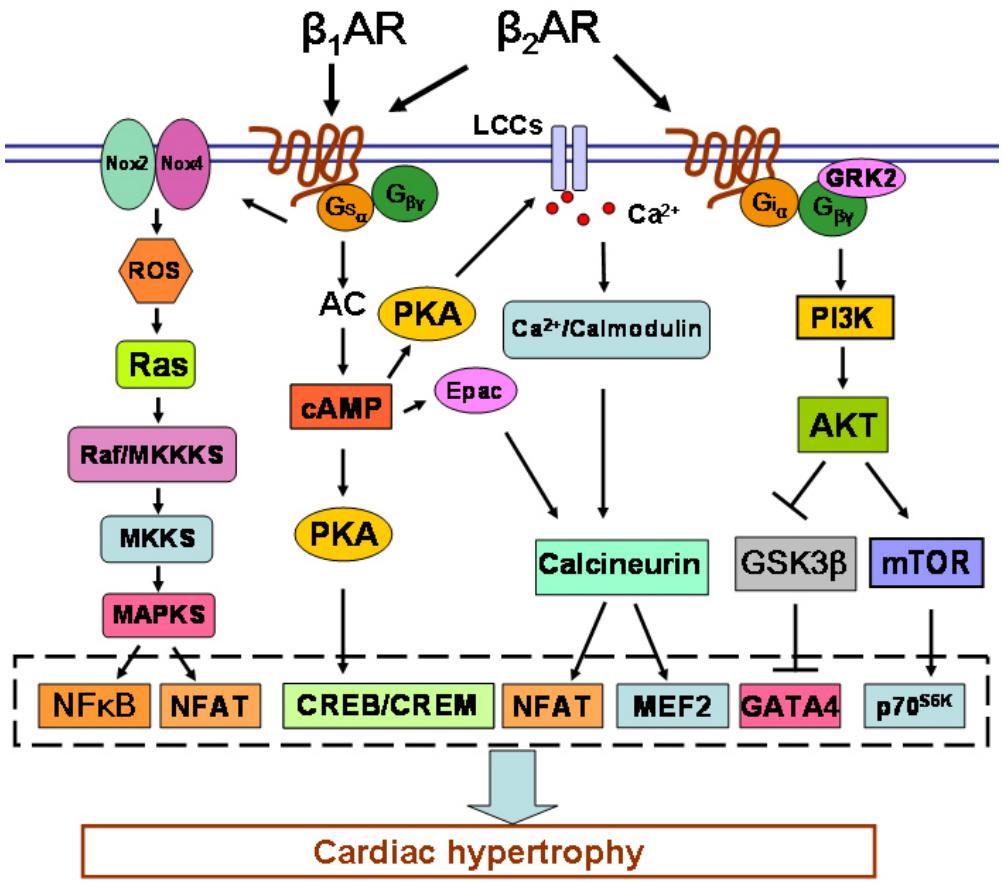

Figure 1. Beta-adrenoceptors signaling pathways mediate cardiac hypertrophy. (1) Beta-ARs mediate the activation of PKA *via* classic signaling pathway, PKA in turn phosphorylates various substrates including the transcription factor CREB/CREM; (2) Beta-ARs increase the activation of NAD(P)H oxidase, a major source of cellular ROS, then lead to the activation of MAPKs cascades including ERK1/2, p38 kinase, JNK, which mediate activation of many transcription factors; (3) Beta-ARs mediate the phosphorylation of LCCs and RyRs, resulting in the increase of intracellular Ca^{2+}, which lead to activation of calcineurin and CaMKII. Calcineurin activates transcription factors such as NFAT, MEF2; (4) $Beta_2$-ARs increase PI3K activity and AKT phosphorylation by coupling to G_i. AKT increases mTOR activity and inhibits GSK-3beta activity, which leads to an increase in transcription of GATA4 and $p70^{S6K}$.

decrease in $beta_1$-AR density in patients or animal models of heart failure, while the density of $beta_2$-AR is unchanged. In that case, the ratio of $beta_2$-ARs/$beta_1$-ARs is increased. This change in receptor density indicates that $beta_2$-AR signaling might play a more important role during cardiac hypertrophy and heart failure develops (61, 62).

Experiments demonstrated that PI3K activity is essential for both basal cell growth and adaptive/maladaptive hypertrophy (63-68). Overexpression of a constitutively active PI3K in transgenic mice resulted in cardiac hypertrophy characterized by increased cardiomyocyte size, whereas overexpression of dominant-negative PI3K led to significant reduction in both body size and heart size in transgenic mice (64). The mechanism by which the PI3K cascades induce cardiac hypertrophy might be contributed to enhancement of the activity of AKT, which phosphorylates mammalian target of rapamycin (mTOR) and inhibits glycogen synthase kinase-3 beta (GSK3beta) signaling in the heart (Figure 1).

Current studies showed that PI3Kalpha and PI3Kgamma are strongly associated with cardiac hypertrophy. These two kinases are composed of a regulatory subunit and a catalytic subunit (69, 70). Several pieces of evidence indicate that PI3Kalpha plays a critical role in adaptive growth and its activity is increased in response to various physiological stimuli including exercise and IGF-1(63, 65). On the other hand, PI3Kgamma/AKT might be involved in GPCR-induced pathological hypertrophy (66). Pressure overload selectively activated p110gamma without affecting p110alpha in the case of pathological hypertrophy (67, 68). The lack of PI3Kgamma inhibited activation of AKT and ERK1/2 signaling pathways and attenuated hypertrophic response in cardiomyocytes to beta-AR stimulation (66). Moreover, PI3Kgamma is involved in down-regulation of beta-ARs under conditions of chronic catecholamine stimulation, yet cardiac-specific overexpression of a catalytically inactive mutant of PI3Kgamma (dominant-negative-PI3Kgamma) was observed to prevent beta-AR down-regulation and desensitization induced by chronic ISO stimulation (71).

3.5. Autocrine/paracrine

Beta-AR activation can promote the production of a number of neurohumoral factors such as Ang II (72), interleukin-6 (IL-6) (73, 74), and endothelin-1(ET-1) (52), which are involved in beta-AR-induced cardiac hypertrophy.

3.5.1. Renin-angiotensin system

The renin-angiotensin system (RAS) has a prominent role in cardiac hypertrophy during heart failure development, since increased cardiac Ang II levels could lead to cardiomyocyte hypertrophy both *in vitro* and *in vivo* (75-77). Experiments showed that all components required for Ang II production were available in the heart, and the formation of cardiac Ang II seemed to be regulated independently of circulating RAS (78, 79). Indeed, the excessive stimulation of beta-AR in the heart induces myocardial hypertrophy with an increase in both cardiac Ang II production and angiotensin converting enzyme (ACE) activity, which does not depend on the increase in peripheral vascular resistance (80). In addition, lack of $alpha_{2A}$/$alpha_{2C}$AR results in chronic sympathetic hyperactivity and elevated Ang II level in the transgenic mouse heart. These mice developed cardiac hypertrophy, and the detrimental cardiac effects could be counteracted by ACE inhibitor treatment (81). These experiments suggest that the secretion of Ang II may be a key mediator in cardiac hypertrophy induced by beta-AR stimulation.

3.5.2. IL-6 /STAT3 signaling pathway

It was reported that IL-6 cytokine level is elevated in heart failure patients and that IL-6 is a strong prognostic marker for the morbidity and mortality of patients with heart failure or after myocardial infarction (82). IL-6 regulates the Janus kinases (JAK)/signal transducers and activator of transcription (STAT) signaling pathway *via* the gp130 receptor system (83). It has been demonstrated that the IL-6/STAT3 signaling pathway might be involved in cardiac hypertrophy. We found that the IL-6/gp130/STAT3 signaling pathway mediated beta-AR-induced atrial natriuretic factor expression in cultured neonatal rat cardiomyocytes (84). Another study reported two different actions of IL-6 in adult rat cardiomyocytes: inducing STAT3 phosphorylation and activating cellular markers of cardiac hypertrophy in the presence of its soluble receptor, activating a separate pathway leading to the phosphorylation of ERK1/2, AKT, and S6 kinase in the absence of its soluble receptor, which has a heart protective effect (85).

We found that ISO treatment can significantly increase IL-6 levels in serum and mouse myocardium. And ISO-induced IL-6 is primarily from cardiac fibroblasts rather than cardiomyocytes. The results suggested that IL-6 mediates ISO-induced delayed STAT3 activation in mouse heart (73). We further found that ISO-induced secretion of IL-6 was mainly mediated by $beta_2$-AR-Gs-AC-cAMP signaling cascade and could be negatively regulated by Gi and PI3K. However, the downstream pathway is not classical PKA but p38 MAPK which mediates $beta_2$-AR-induced IL-6 production in neonatal mouse cardiac fibroblasts (74).

3.6. Novel signaling pathways

3.6.1. Epac signaling pathway

Stimulation of beta-AR induces production of cAMP, which could activate exchange protein directly activated by cAMP (Epac) as well as PKA. Epac is subdivided into Epac1 and Epac2, both characterized by a regulatory domain directly binding to cAMP and a catalytic region containing an exchange factor motif that catalyzes the exchange of GDP from GTP on Rap GTPases (86). Experiments demonstrated that Epac might participate in regulation of cardiac hypertrophy. Cardiomyocyte hypertrophy was found induced by a selective Epac activator (8-CPT) and by Epac protein overexpression after transfection by an adenovirus encoding Epac1 (87). Epac activation leads to cytoskeletal reorganization, increases in protein synthesis, and expression of markers of cardiac hypertrophy. In addition, the expression of Epac1 was increased in hypertrophic myocardium (87). A recent study showed that Epac1 greatly contributed to beta-AR-induced myocyte hypertrophy. Silencing Epac1 expression blocked the hypertrophic effect of ISO *in vitro* (88). Epac activated a pro-hypertrophic signaling pathway independent of its classic effector, Rap1, but involving the small GTPase Ras, the phosphatase, calcineurin, and Ca^{2+}/ CaMKII (88) (Figure 1).

3.6.2. Endocytosis signaling pathway

Following exposure to chronic ISO stimulation, the endocytosis of beta-AR is triggered which turns off the downstream signaling pathways, avoiding excessive stimulation. This process of endocytosis is accompanied by a series of signal transduction. Previous studies showed that endocytosis of beta-ARs induced activation of MAPK, including p38 kinase and ERK1/2 (42, 89). Morisco *et al.* (90) reported that $beta_1$-AR underwent prolonged endocytosis (>4 hours) after ISO stimulation. Inhibition of the endocytosis blocked ISO-induced AKT activation, attenuated myocyte hypertrophy and ANF transcription (90). These results suggested that activation of the endocytosis is required for ISO-induced cardiac hypertrophy.

4. BETA-ADRENOCEPTORS AND CARDIAC FIBROSIS

Cardiac fibroblasts account for about two thirds of the total cells in the heart and are crucial to normal myocardial function. However, Cardiac fibroblasts are also involved in the adverse cardiac remodeling, which accompanies with hypertension, myocardial infarction, and heart failure.

Cardiac fibroblasts expressed $beta_2$-AR but not $beta_1$-AR, which was verified by many independent studies. With radioligand binding assay to detect the expression of subtypes of beta-ARs, we demonstrated that the affinity to ICI 118551 (a selective inhibitor of $beta_2$-AR) was higher than that to CGP20712A (a selective inhibitor of $beta_1$-AR) in neonatal rat cardiac fibroblasts (91). It suggests that $beta_2$-AR is the preponderant beta-AR subtype in cardiac fibroblasts. Reverse transcription PCR also revealed high-

level expression of $beta_2$-AR in adult rat cardiac fibroblasts (92), and adult human cardiac fibroblasts (93), with little or no $beta_1$-AR detected. Thus, the effects of catecholamine on cardiac fibroblasts may be mediated predominantly *via* activation of $beta_2$-AR.

4.1. $Beta_2$-AR and cardiac fibroblast proliferation

It has been observed that $beta_2$-AR stimulation could induce cardiac fibroblast proliferation *in vitro* (88, 90), yet the mechanism remains unclear. Proliferation signals such as ERK1/2 and p38 MAP kinase activation were observed after $beta_2$-AR stimulation. In addition, cardiac fibroblasts secrete many cytokines in response to $beta_2$-AR stimulation such as ET-1, IL-6, and transforming growth factor-$beta_1$ (TGF-$beta_1$). Turner *et al.* (94) demonstrated that $beta_2$-AR-mediated human cardiac fibroblast proliferation was dependent on autocrine of ET-1.

4.2. $Beta_2$-AR and collagen synthesis

The development of cardiac fibrosis is always accompanied with increased collagen synthesis, and the ability of beta-AR stimulation to influence extracellular matrix (ECM) protein expression by cardiac fibroblasts remains unclear. There is a little evidence about the increase of ECM protein induced by beta-AR stimulation *in vitro*. Conversely, there was a study showed that beta-AR activation inhibited TGF-$beta_1$-stimulated collagen synthesis *via* inhibiting ERK 1/2 and Smad signaling in cardiac fibroblasts (95).

4.3. Beta-AR stimulation induced cardiac fibrosis *in vivo*

A series of experiments showed that both mice and rats developed cardiac fibrosis after a moderate or a high dose of ISO injection for one or two weeks *in vivo* (96, 97). Nakatsuji *et al.* (98) reported that alpha-smooth muscle actin-expressing cardiac myofibroblasts were observed at the border of the injured myocardium 3-7 days after ISO injection. Benjamin *et al.* (99) purported the cardiac fibroblast proliferation in ISO-treated hearts to be a direct response to ISO rather than a result of myocyte loss. Although $beta_2$-AR stimulation induced cardiac fibroblast proliferation *in vitro*, cardiac fibrosis was not observed *in vivo* after $beta_2$-AR stimulation (100, 101), indicating that apart from cardiac fibroblasts, cardiomyocytes also played a key role in the regulation of cardiac fibrosis.

4.4. Cell-cell communications in beta-AR-mediated cardiac fibrosis

Although cardiac fibroblasts are pivotal in the progress of cardiac fibrosis, the specific overexpression of some pro-hypertrophic genes in cardiomyocytes such as G_s (27) could also result in cardiac fibrosis. Both the transgenic mice with specific overexpression of $beta_1$-AR and those with $beta_2$-AR under the control of the alpha-myosin heavy chain promoter developed cardiac fibrosis (24-26), with no changes in the amount of $beta_2$-AR in cardiac fibroblasts. Besides, stimulation with a selective $beta_1$-AR agonist (dobutamine) could also induce cardiac fibrosis (22). The mechanism is not fully understood and may be attributed to the communications between myocytes and fibroblasts.

The paracrine activities of cardiomyocytes are crucial for regulating the function of cardiac fibroblasts. Stimulation of the beta-AR in cardiomyocytes can induce secretion of cytokine and growth factor including Ang II, endothelin, and TGF-beta (102, 103), which in turn exert a profibric effect on fibroblast. There is also a direct cell-cell communication between cardiomyocytes and cardiac fibroblasts. Recently studies suggested that every cardiomyocyte was in direct contact with one or more fibroblasts (104). That viewpoint was confirmed by a series of experiments: Vatner *et al.* (105) generated chimeric mice that carried both overexpressing G_s protein cells and wild-type cells. The chimeric mice developed cardiac fibrosis, and importantly most of the clustered fibrosis was found to be surrounding cardiomyocytes carrying the overexpressing G_s protein. Besides paracrine and mechanical force, Cardiac fibroblasts would also connect to cardiomyocytes by connexins, which play a key role in the regulation of the electrical activity of heart (106). However, the effect of these connections on beta-AR-mediated cardiac fibrosis remains elusive.

5. BETA-ADRENOCEPTORS AND CARDIOMYOCYTE APOPTOSIS

Many studies in both humans and animals have demonstrated that myocyte apoptosis is associated with a variety of cardiac pathological states including heart failure (107). In cultured neonatal rat cardiomyocytes, norepinephrine-induced myocyte apoptosis was abolished by the beta-AR antagonist propranolol, and mimicked by both ISO and cAMP analogue, 8-Br-cAMP (108). In addition, the ISO-induced neonatal rat cardiomyocyte apoptosis was completely inhibited by PKA inhibitor KT5720 (108). These findings suggest that cAMP/PKA pathway is necessary to mediate cardiomyocyte apoptosis induced by beta-AR agonist. Both mice overexpressing $beta_1$-AR (109) and G_s (110) in the myocardium exhibited cardiomyocyte apoptosis and developed heart failure. Sustained $beta_1$-AR stimulation promotes apoptosis of myocardial cells, whereas sustained $beta_2$-AR stimulation protects myocytes against apoptosis through G_i/PI3K pathway (6, 8). An experiment with adult rat cardiomyocytes suggested that beta-AR stimulation increased cytochrome c release and decreased mitochondrial membrane potential through ROS/JNK pathway (40). Further study found that Rac1 activation is required for myocyte apoptosis and results in the activation of JNK and mitochondrial death pathway (111).

In the condition of sustained $beta_1$-AR stimulation, disturbance of Ca^{2+} handling was noticed (112). Ca^{2+}-induced CaMKII activation has been shown to be an important PKA-independent mechanism in $beta_1$-AR-induced cardiomyocyte apoptosis *via* the primary mitochondrial death pathway (113, 114). On the other hand, *in vivo* studies suggest that CaMKII inhibition protects against cardiomyocyte apoptosis and cardiac remodeling from enhanced beta-AR stimulation (115, 116).

Increase of intracellular Ca^{2+} induced by beta-AR stimulation also results in calcineurin activation. Saito *et al.* (117) found that both nifedipine (a LCCs antagonist) and calcineurin inhibitors could suppress ISO-induced cardiomyocyte apoptosis. ISO stimulation induced dephosphorylation of a pro-apoptotic molecule Bad which promotes cell death and cytochrome c release from mitochondria to cytosol, both required for calcineurin activation. These results suggest that beta-AR activation induces apoptosis through Ca^{2+}/calcineurin pathway. However, another study has reported that the activation of calcineurin protected cardiomyocytes against apoptosis induced by 2-deoxyglucose, staurosporine, and ischemia-reperfusion (118). The calcineurin activation has also been reported to be required for the inhibitory effect of endothelin-1 on oxidant stress-induced cardiomyocyte apoptosis (119). These findings suggest that the calcineurin pathway might be either pro- or anti-apoptotic depending on the stimuli. Under beta-AR activation, the effect of this pathway is pro-apoptotic.

Another pro-apoptotic factor in beta-AR-stimulated apoptosis is matrix metalloproteinase-2 (MMP-2). Beta-AR stimulation increases the expression and activity of MMP-2, and inhibits tissue inhibitor of metalloproteinase-2 (TIMP-2) expression, possibly through JNK activation (120). MMP-2 interacts with $beta_1$- integrins and interferes with $beta_1$-integrin-mediated survival signals, leading to JNK activation and mitochondrial death pathway (120). Intact $beta_1$-integrin protects cardiomyocytes against beta-AR-stimulated apoptosis and cardiac remodeling (121, 122), but it is cleaved under beta-AR stimulation, and its cytoplasmic domain has been shown to induce apoptosis in adult cardiomyocytes (123). MMPs inhibitor, on the other hand, could suppress $beta_1$-integrin shedding in cardiac fibroblasts (124). These findings suggest that MMP-2-induced apoptosis might involve integrin fragmentation during beta-AR stimulation. A recent study reported that glycogen synthase kinase-3beta (GSK-3beta) plays a key role in beta-AR/MMP-2 induced apoptosis and $beta_1$-integrins involved anti-apoptotic effect. The pro-apoptotic effect of GSK-3beta may be mediated *via* its nuclear localization and induction of pro-apoptotic genes such as Gadd153. This activity of GSK-3beta activity could be inhibited by $beta_1$ integrin through PI3K pathway (125).

Notably, beta-AR activation does not always result in detrimental effects on the heart. A recent study showed that $beta_1$-AR and $beta_2$-AR activation increased the secretion and uptake of ubiquitin in adult rat ventricular myocytes possibly *via* the AC pathway, the increased extracellular ubiquitin in turn inhibits the pro-apoptotic effects of beta-AR stimulation (126). The mechanism involves activation of the PI3K/AKT pathway, resulting in the inactivation of GSK-3beta/JNK and mitochondrial pathways. This negative feedback protects cardiomyocytes from beta-AR-stimulated apoptosis in a paracrine manner.

6. CONCLUSIONS

During the process of heart failure, it is now known that chronic beta-AR activation plays a key role in pathological cardiac remodeling. Exploring and characterizing of the beta-AR signaling pathways could help us to find a way to reverse the pathological remodeling and to improve cardiac function. Recently, various new methods have been applied to this field of study such as transgenic technology, which greatly promoted the comprehensive understanding of the beta-AR signaling pathways. More and more new signaling pathways have been discovered with diverse effects in the beta-AR-mediated cardiac remodeling, some of which may become therapeutic targets for new drugs.

7. ACKNOWLEDGEMENTS

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Abbreviations: adrenoceptors: ARs; G protein-coupled receptor: GPCR; protein kinase A: PKA; L-type calcium channels:LCCs; ryanodine receptors: RyRs; phosphatidylinositol 3-kinase: PI3K; sarcoplasmic reticulum: SR; isoproterenol: ISO; cAMP response binding protein: CREB; atrial natriuretic factor: ANF; mitogen-activated protein kinase: MAPK; reactive oxygen species: ROS; epidermal growth factor receptor: EGFR; nuclear factor of activated T-cells: NFAT; calmodulin-dependent protein kinase II: CaMKII; insulin-like growth factor 1: IGF-1; Akt/PKB: AKT; mammalian target of rapamycin: mTOR; glycogen synthase kinase-3beta: GSK3beta; angiotensin II: Ang II; interleukin-6: IL-6; endothelin-1: ET-1; Janus kinases: JAK; signal transducers and activator of transcription: STAT; exchange protein directly activated by cAMP: Epac; TGF: transforming growth factor; extracellular matrix: ECM; matrix metalloproteinase-2: MMP-2; tissue inhibitor of metalloproteinase-2: TIMP-2

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