Chapter 16
Role of β-Adrenoceptor/Adenylyl Cyclase System in Cardiac Hypertrophy

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Abstract Activation of the sympathetic nervous system (SNS) releases norepinephrine, stimulates β-adrenoceptors, and plays a crucial role in the development of cardiac hypertrophy upon activation of various cellular signaling pathways in the heart. At early stages, norepinephrine-induced cardiac hypertrophy serves as an adaptive mechanism for maintaining heart function whereas at late stages, it is associated with contractile dysfunction, alterations in electrical activity, and programmed cell death. Activation of G_s-protein coupled β_1- or β_2-adrenoceptors produces an increase in cardiac contractility and some deleterious effects whereas that of G_i-protein coupled β_2- or β_3-adrenoceptors is known to result in beneficial adaptive actions in the heart. While the increase in G_s-protein involves the downstream activation of adenylyl cyclase (AC), the activation of G_i-proteins is associated with either a depression in AC or augmentation of guanylate cyclase activity. In this article, we discuss the physiological aspects of β-adrenergic signaling pathways and their modification in the hypertrophied heart as well as their participation in the transition of cardiac hypertrophy to heart failure. Furthermore, we highlight the actions of some components of the β-adrenoreceptor signaling cascade that may participate in the genesis of cardiac hypertrophy and thus serve as pharmacological targets for the prevention of cardiac hypertrophy or treatment of the hypertrophied failing heart.

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16.1 Introduction

Cardiac hypertrophy develops as a consequence of physiological or pathological stimulus, which induces activation of different signal transduction pathways to increase muscle mass. Since adult myocardial cells are terminally differentiated, cardiac growth during the hypertrophic process is due to an increase in the size of individual myocardial cells with little or no change in cell number [1]. In highly trained athletes, the growth of the cardiac ventricle refers to physiological hypertrophy, which is not associated with any predisposition to heart failure. In contrast, pathological hypertrophy of mechanically overloaded heart is linked with specific changes that make the heart more sensitive to injury. It is noteworthy that pathological hypertrophy at initial stages is a physiological response of the heart to an increased workload. However, at late stages the heart is unable to maintain cardiac function and exhibits certain distinct features of pathological hypertrophy. Although in addition to hemodynamic overload, different circulating hormones including those released upon the activation of sympathetic nervous system (SNS) are known to play important roles in the genesis of cardiac hypertrophy, this review will be focussed to discussion on the involvement of circulating catecholamines in the development of both physiological and pathological forms of hypertrophy.

16.2 Role of the SNS and Catecholamines in Cardiac Hypertrophy

Development of heart hypertrophy is a multifactorial process, which is characterized by an increase in contractile protein content, myofilament organization, and expression of embryonic markers for the genes encoding these proteins [2–4]. Furthermore, in addition to mechanical factors, different hormones play a crucial role in the genesis of cardiac hypertrophy. In this regard, catecholamines are considered as “hypertrophy hormones” and the activation of SNS and subsequent elevations in circulating catecholamines contribute to changes in cardiac function [5, 6]. Although these alterations in the SNS can be initially considered as compensation for increased hemodynamic load and exhibits functional benefits associated with increased muscle mass, the hypertrophied heart ultimately dilates and fails in a process referred to as decompensation [7, 8]. Indeed, a direct correlation between plasma levels of catecholamines including norepinephrine and mortality in patients with chronic congestive heart failure (CHF) has been documented. In addition to catecholamines *per se*, their oxidation products, such as amino chromes

and adrenolutin, have been suggested as an independent predictor of survival in CHF patients [9]. It should be noted that despite a considerable effort, molecular mechanisms involved in the transition from cardiac hypertrophy to heart failure are incompletely understood. It is therefore, that both pathological states (cardiac hypertrophy and heart failure) with respect to the involvement of the SNS in their pathogenesis are discussed in this article. Furthermore, although both α-adrenoceptors and β-adrenoceptors (β-ARs) are known to mediate cardiomyocyte growth, we will discuss only those mechanisms associated with the β-adrenoceptor complex. In particular, we will focus on the role of adenylyl cyclase (AC) and protein kinase A (PKA) in signaling pathways leading to cardiac hypertrophy.

16.3 AC as a Mediator of the β-Adrenoceptor Signaling Pathway in the Heart

Stimulation of β-ARs leads to the activation of AC. There are three subtypes of β-ARs, designated as β_1-AR, β_2-AR and β_3-AR, identified in the heart, which differ in function as well as molecular and pharmacological characteristics. The β_1-AR is the most abundant subtype expressed within the mammalian heart (about 75–80 % of the total β-ARs), while β_2-ARs account for about 20–25 % of total β-ARs. Expression of β_3-ARs in cardiac tissue is variable across species; low expression levels are found in human myocardium, while these are absent in rats [10, 11]. In addition, localization of the β-AR subtypes is different within the cardiomyocytes with higher concentration of β_2-ARs within caveolae, while most β_1-ARs are distributed throughout the cell membrane [12].

The β_1 and β_2 ARs are normally coupled to *Gs* proteins to stimulate AC activity and generate cyclic AMP (cAMP), a second messenger, which in turn activates PKA. The activated PKA mediates phosphorylation of target proteins involved in metabolic regulation, growth control, muscle contraction, and cell survival or death. The signal transduction mechanisms associated with G_s-protein coupled β_1- and β_2-ARs are depicted in Fig. 16.1. Stimulation of the β-ARs is the most potent mechanism to augment cardiac function in response to "fight -or- flight". As a consequence, there is an increase in cardiac contractility (positive inotropic effect), acceleration of cardiac relaxation (positive lusinotropic effect), and increase in heart rate (a positive chronotropic effect). These effects in cardiac cells upon stimulation of the β-ARs are mediated by phosporylation of Ca^{2+}-handling proteins, including sarcolemmal L-type Ca^{2+} channels, sarcoplasmic reticulum (SR) proteins (phospholamban and ryanodine receptors) as well as myofilament components (troponin I and C proteins).

Several studies have shown that β_2-ARs are coupled to the inhibitory G_i protein under certain conditions, in addition to interacting with *Gs* protein. This leads to attenuation of interaction with AC and activation of an extracellular signal-regulated kinase (ERK) cascade involving Src tyrosine kinases and Ras [13, 14]. The β_2-ARs-G_i signaling has also been demonstrated to induce the recruitment and

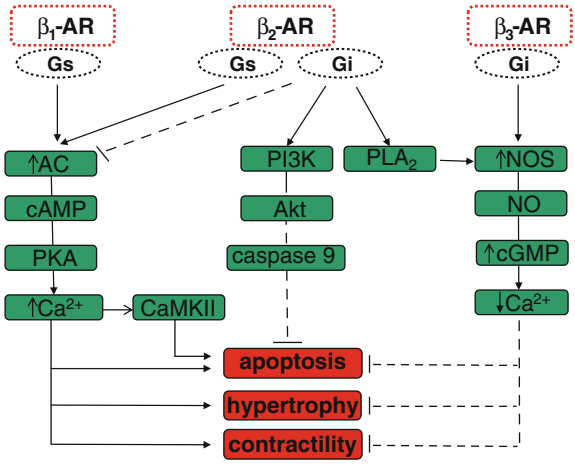

Fig. 16.1 Different signaling pathways activated upon stimulation of different types of *β*-adrenoceptors and their physiological responses. *β*-ARs, *β*-adrenoceptors; G_s, stimulative regulative *G*-protein; G_i, inhibitory regulative *G*-protein; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; Ca^{2+}, calcium; CaMKII, calcium/calmodulin-dependent protein kinase II; PI3 K, phosphatidylinositol 3-kinase; Akt, protein kinase B; PLA_2, phospholipase A_2; NOS, nitric oxide synthase; NO, nitric oxide; cGMP, cyclic guanosine monophosphate

activation of phosphatidylinositol 3-kinase (PI3 K) and the serine-threonine kinase (Akt) which regulates cardiomyocyte growth and apoptosis. Furthermore, stimulation of the β_2-ARs-G_i signaling axis results in the activation of phospholipase A_2 (PLA_2), inhibition of nitric oxide synthase (NOS) activity, alterations in calcium handling, and decreased cardiac contractility [15]. The signal transduction pathway associated with β_2-ARs-G_i-protein complex is shown in Fig. 16.1. In addition, there is evidence that β_2-ARs mediate responses through a G-protein-independent manner. In fact, Na^+/H^+ exchange regulatory factor (NHERF), an inhibitor of Na^+/H^+ exchanger type 3 (NHE3) has been found to bind to the cytoplasmic domain of the β_2-ARs resulting in a release of the inhibitory effects of NHERF on NHE3 [16]. These findings provide a putative mechanism by which the the β_2-ARs exert opposing effects on NHE3 activity and thus induce alkalization. However, there is no clear evidence for a catecholamine-dependent inotropic response due to β_2-ARs-dependent alkalization. As shown in Fig. 16.1, β_3-ARs have also been implicated as inhibitors of contractile function. In fact, coupling of β_3-AR to G_i-proteins has been shown to lead to activation of endothelial NOS expressed in ventricular myocytes and production of NO, resulting in the synthesis of cGMP and consequent reduction in cardiac contractility [17]. Interestingly, overexpression of β_3-ARs in transgenic mice has been found to increase cardiac contractility in heart via coupling to G_s-proteins [18]. Thus, a great deal of information needs to be obtained concerning the β_3-AR associated signal transduction mechanisms for making a meaningful conclusion.

16.4 Pathological Aspects of Sustained Stimulation of the β-Adrenergic Receptors

16.4.1 Cellular Growth and Apoptosis Regulated by Stimulation of β-ARs

It is well known that receptors for different hypertrophic agents including angiotensin II, endothelin-1, and α-AR agonists are coupled to G_q-proteins. However, D'Angelo et al. [19] have also suggested a link between β-ARs and G_q-proteins. Indeed, β-AR insensitivity is now considered to be a hallmark of the G_q-phenotype. Impaired G_s-coupling attributed to PKC-mediated receptor phosphorylation, increased G_i-protein content, and decreased AC content are observed in the G_q-heart. These findings have raised an important question of whether restoration of β-AR responsiveness can rescue cardiac contractility in $G\alpha_q$-mice. Genetically modified G_q-mice overexpressing β-ARs experienced 30-fold enhanced basal left ventricular function, and diminished fetal cardiac gene expression. In case of 140-fold increase of β-ARs, ventricular function, morphometric hypertrophy and fetal gene expression were exacerbated in G_q-mice. Consequently, most of these mice died at 5 weeks of a rapidly progressive dilated cardiomyopathy. Based on these findings, it is apparent that G_q-mediated hypertrophy exhibits multiple molecular defects in β-AR signaling and that normalization of cardiac contractility through restoration of β-AR responsiveness increases myocardial fibrosis, and lead to the development of heart failure [20–22].

Although β_1-ARs are the predominant β-AR subtype in mammalian ventricular cardiomyocytes, and that β_2-AR-dependent signal may only represent a relatively minor component of catecholamine response under normal physiological conditions, the β_2-AR linked mechanisms become more relevant with respect to contractile function in the hypertrophied and failing heart [23, 24]. Of note, we have shown that the volume overload-induced changes in the different components of β-AR complex in cardiac hypertrophy due to arteriovenous shunt are gender dependent [25]. In addition, the expression of β_2-ARs has been found to be up-regulated in denervated, transplanted human hearts [26]. Clinical studies have shown that non- selective β-blockers reduce sudden cardiac death in post-myocardial infarction patients, while selective β_1-AR blockers did not exert such a beneficial action [27]. Subsequently, studies employing transgenic mice have shown that overexpression of β_1-ARs results in a dilated cardiomyopathy phenotype, even in young mice, whereas mice overexpressing β_2-ARs (up to 150-fold over endogenous receptor expression) did not exhibit any significant cardiac pathology [28, 29]. Furthermore, cardiac function in mice with mild overexpression of β_2-ARs has been shown to be enhanced, perhaps as a result of the dual coupling of these receptor subtypes [30]. On the other hand, higher overexpression of β_2-ARs (>200-fold) resulted in a similar negative effect on heart function as the overexpression of β_1-ARs [31]. The hypothesis that the β_1-ARs are the predominant mediators of catecholamine-induced chronotropy and inotropy is

also supported by evidence that β_1-AR knockout in mice is generally embryonic lethal, while β_2-AR knockout mice are healthy and the physiological consequences are only observed under conditions of stress and exercise [32, 33].

Although α-ARs were believed to be main mediators of growth-promoting signaling, there is now evidence that β-ARs induce expression of proto-ocogenes (c-fos, c-jun), expression of the hypertrophic marker, atrial natriuretic factor (ANF), and stimulate protein synthesis in cardiomyocytes [34, 35]. A wide variety of calcium-dependent kinases and phosphatases have been implicated in signal transduction leading to transcriptional activation and/or hypertrophic growth responses [36, 37]. It has been suggested that the β_1-AR hypertrophic effects are not associated with either the activation of cAMP/PKA, or ERK activation [38, 39], but requires Akt-glycogen synthase kinase 3b (GSK-3b)-GATA4-signaling and activation of tyrosine kinase. In contrast, β_2-AR stimulation has been shown to inhibit β_1-AR mediated cardiomyocyte hypertrophy [38].

The β_1- and β_2-ARs differ in their role in apoptotic cell death, a process thought to be important in the transition from cardiac hypertrophy to heart failure. Stimulation of the β_1-ARs leads to apoptosis, while stimulation of the β_2-ARs may produce anti-apoptotic signals. Several studies have reported that the cAMP/PKA signaling pathway plays a critical role in β_1-AR-mediated apoptotic cell death. This was confirmed by the finding that a PKA inhibitor, H-89, was capable of abolishing programmed cell death [40, 41]. On the other hand, although the β_2-AR stimulation enhances cAMP formation, it elicits cardioprotective effects [40, 42]. It is noteworthy that some studies have reported that apoptosis due to sustained β_1-AR stimulation is PKA independent. In fact, recent studies have demonstrated that activation of calcium/calmodulin-dependent protein kinase II (CaMKII) due to β_1-AR stimulation constitutes a novel PKA-independent pro-apoptotic stimulus [43]. In addition, the expression of ANF was found to be correlated to the activation of CaMKII [44]. However, the exact molecular mechanisms underlying PKA-independent activation of CaMKII leading to apoptosis during sustained β_1-AR stimulation remains to be explored.

The main anti-apoptotic mechanism of the β_2-AR has been suggested to be linked to PI3 K-Akt signal transduction. Overexpression of PI3 Kα has been demonstrated to increase cardiac hypertrophy, while deletion of the PI3 Kα regulatory subunits attenuates myocardial hypertrophy in response to exercise training [45]. Concurrently, chronic activation of Akt leads to a decrease in PI3 K activity at the cell membrane due to a negative feedback mechanism involving insulin receptor substrate (IRS)-1 and 2. This molecule binds to the regulatory subunit of PI3 Kα and recruits it to the activated growth hormone receptor. Of note, Akt-mediated degradation and decreased synthesis of IRS-1 and -2 prevents recruitment of PI3 Kα to the cell membranes, which consequently attenuates the PI3 Kα-mediated beneficial effects; however, introduction of constitutively active PI3 K to failing hearts restores cardiac function and decreases ischemia/reperfusion injury [46]. In addition to this mechanism, the β-AR-mediated survival signal was found not only to counteract the concurrent G_s-induced apoptosis, but also to protect cardiomyocytes against a wide range of apoptotic insults, such as due to reactive oxygen species [42].

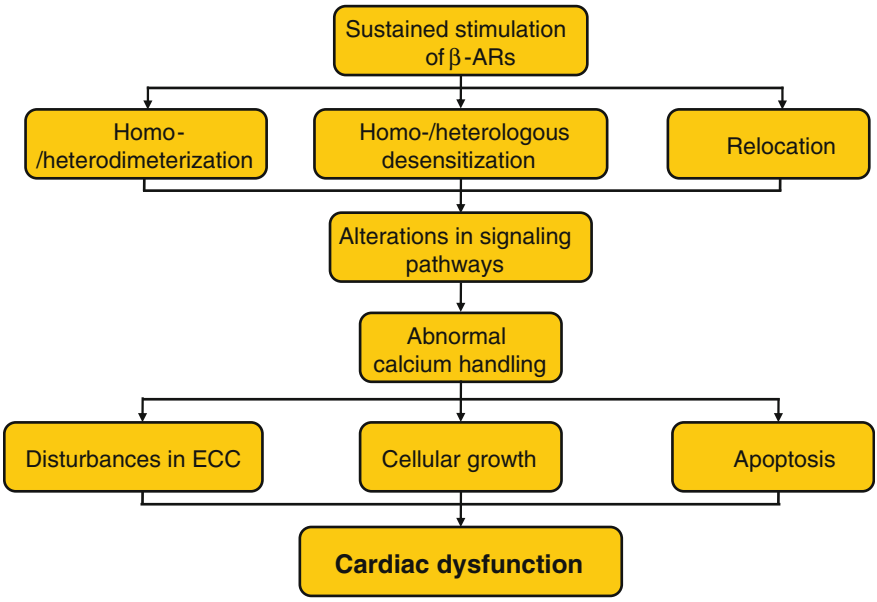

Fig. 16.2 Consequences of sustained stimulation of beta-adrenergic receptors leading to alterations in signaling pathways and modification of cardiac function. Excitation–contraction coupling (*ECC*)

16.4.2 Dimerization, Desensitization, and Relocalization of β-ARs

While the SNS activation provides essential inotropic support, sustained AR stimulation by catecholamines leads to β-AR dimerization and desensitization culminating in alterations in receptor responsiveness (Fig. 16.2). β-AR homo- and heterodimerization leads to altered cardiomyocyte responsiveness due to switching of *G*-protein coupling. In fact, β_1/β_2-ARs dimers are linked to G_s-proteins and produce synergic effects with respect to production of cAMP and increased cardiac contractility compared with either receptor alone [47]. While both β_2- and β_3-ARs can couple with G_i-proteins alone, heterodimers are unable to produce signal through G_i-proteins [48]. This β-AR association may help to understand the pathophysiology of cardiac hypertrophy as well as to explain the effectiveness of selective/non-selective β-AR agonists/antagonists.

Another consequence of sustained β-adrenergic stimulation is desensitization, which can be often seen in various G-protein coupled receptors (GPCRs). If it occurs in the context of diminished responsiveness to a diverse array of other agonists for GPCRs, it is referred to as heterologous desensitization and results from a negative feedback regulation by PKA-mediated phosphorylation. In addition to phosphorylation of the β-AR itself, phosphorylation of downstream protein targets such as *G*-proteins, and AC, also occurs. Furthermore, switching the

expression of *G*-proteins in favor of G_i-proteins over G_s-proteins also decreases β-AR-mediated response resulting in an attenuation of AC activation [49]. Likewise, induction of cAMP-hydrolyzing phosphodiesterases (PDEs) also blunts the effects of β-AR-stimulated cAMP signalling in cardiomyocytes producing a decrease in the SNS control of cardiac output [50]. Homologous or agonist-specific desensitization occurs during chronic exposure to an agonist resulting in a diminished response to that specific agonist. This type of desensitization is generally mediated by GPCR kinase family (GRK1-6), particularly GRK2, which is also known as β-AR kinase (β-ARK1) [51]. GRKs phosphorylate the activated ARs to alter their conformation and prevent further Signallng via G_s-proteins [52].

Early findings from experimental studies using the isoproterenol-induced cardiac hypertrophy model have suggested that a reduction in β-AR-mediated contractile response at the initial stage of cardiac hypertrophy is attributed to post-receptor modifications of the β-AR-cAMP signaling pathway rather than down-regulation of the myocardial β-ARs *per se*. In fact, short-term isoproterenol exposure produced a 53 % reduction in β-AR-mediated stimulation of AC, but the content of these receptors was unchanged [53]. Such a reduction in AC activity was shown under basal conditions and upon stimulation by β-AR agonists as well as non-adrenergic stimuli such as forskolin, sodium fluoride, and GTP [54–57]. Furthermore, since responses to these stimuli were reduced by various substances, it would appear that heterologous rather than homologous desensitization of AC was induced. In accordance to this, molecular studies have revealed that mRNA levels of AC are decreased in hearts chronically exposed to isoproterenol [57]. Desensitization of AC upon stimulation of the β-ARs is attributed to the increased activity of inhibitory G-proteins, as increased G_i-protein α-subunit mRNA level was found [58, 59]. On the other hand, the alterations in G_i- subunit mRNA levels were prevented by β-AR blockade [60]. Based on these results, it has been suggested that the increased functional activity of inhibitory G_i-proteins contributes to reduced β-AR-mediated inotropic responses upon chronic β-AR-stimulation. Of note, β_3-ARs are unlikely to undergo desensitization; in fact they are desensitization-resistant [61]. In contrast, the data regarding alterations in G_s-protein in hearts exposed to β-AR agonists are not clear as reduced level of G_s-proteins has been reported, while others have observed that reduced β-AR-mediated AC stimulation originates from decreased expression of G_s-proteins [62–64].

Sustained SNS stimulation, results in downregulation of the β-ARs due to redistribution of the receptors from the cell membrane to endosomal compartments (relocalization) and thereby it leads to an overall reduction in the number of receptors available for activation at the cell surface [65]. Despite numerous investigations exploring the mechanisms responsible for β-ARs desensitisation, the question of whether chronic β-AR desensitization in heart failure is considered as a deleterious or beneficial adaptive mechanism remains unanswered. From studies employing human tissue and a wide variety of models of experimental cardiac hypertrophy or heart failure, there is a general consensus that both pathologies are associated with alterations in the β-ARs. With respect to cardiac hypertrophy, it has been suggested that alterations in β_1-AR signal transduction

can be dependent on the type and stage of heart hypertrophy. In view of the activation of the SNS and renin-angiotensin-aldosterone system (RAAS), it appears that compensated and decompensated forms of cardiac hypertrophy may depend on the duration of exposure of the heart to different humoral and other growth factors circulating in the body. It has been shown that β-AR-mediated signal transduction mechanisms are up-regulated or unchanged in the compensated stages of cardiac hypertrophy, while these are down-regulated in the decompensated stages of cardiac hypertrophy [66]. Such findings are in line with the hypothesis that upregulation of β-AR mechanisms in compensated cardiac hypertrophy may play an adaptive role in maintaining heart function, while down-regulation of these mechanisms in decompensated cardiac hypertrophy may reflect the loss of its support to the failing heart. In a series of studies using various models of heart failure, it has been documented that β_1-ARs are downregulated, while β_2-AR expression is preserved [67, 68]. Furthermore, the coupling of both β-AR subtypes has been found to be impaired in human failing heart, presumably as a result of the up-regulation of GRK, namely GRK2 and/or GRK5 [69].

Although activation of the SNS plays a critical role in the pathogenesis of cardiac hypertrophy, excessive amount of circulating catecholamines may serve as a trigger for the transition of myocardial hypertrophy to heart failure. In this regard, treatment of rats for 24 h with a high dose (40 mg/kg) of a synthetic catecholamine, isoproterenol has been shown to result in increased LVEDP, depressed rates of pressure development and pressure decay as well as intracellular Ca^{2+}-overload [70]. Excessive amounts of catecholamines released from sympathetic nerve endings as well as from the adrenal medulla under stressful conditions are considered to also produce intracellular Ca^{2+}-overload and cardiac dysfunction through the β_1-AR signal transduction pathway [71]. Interestingly, β-AR kinase (GRK2) has been suggested to be a key molecule in the transition of myocardial hypertrophy to heart failure [72]. In patients with dilated cardiomyopathy the β-adrenergic responsiveness of the myocardium is diminished. It was shown that in these patients the expression of the β_1-AR is reduced at the mRNA and protein level whereas the expression of the inhibitory G_i- protein is increased. Furthermore, the expression of the GRK is elevated and induces uncoupling of the β_1-ARs. These alterations of the β_1-AR signal cascade may be induced by an elevated catecholamine release in patients with dilated cardiomyopathy [73]. Thus, excesses in catecholamines may be viewed as a mechanism for the transition of myocardial hypertrophy to heart failure. Indeed, it is likely that a threshold catecholamine concentration triggers this maladaptive response.

Recent studies have found that polymorphism of β_2-ARs may be a disease modifier. In fact, changes in the coding sequence of the β_2-AR gene (where isoleucine is substituted for threonine at 164 amino acid in the fourth transmembrane-spanning domain) have been suggested not to predispose heart failure, but individuals with heart failure harbouring this receptor are at significant risk for rapid decompensation [74]. This polymorphism leads to a decrease in the basal and agonist-stimulated AC activities as a result of defective coupling of the receptor to G_s-proteins [75]. It is plausible that other polymorphism processes may underlie

Table 16.1 Alterations in β-adrenoceptor complex in cardiac hypertrophy progressing into heart failure

Density of β-ARs	Healthy heart $\beta_1 \gg \beta_2$	Diseased heart $\beta_1 \cong \beta_2$
Localization of β-ARs	Membrane surface $\gg$ caveolae	Membrane surface $\ll$ caveolae
Coupling of β_2-ARs	$G_s \gg G_i$	$G_i \gg G_s$
Adenylyl cyslase isoforms	AC type V $\cong$ AC type VI	AC type V $\gg$ AC type VI

β-ARs β-adrenoceptors
AC adenylyl cyclase
G_s stimulative regulative *G*-protein
G_i inhibitory regulative *G*-protein

inter-individual variations in catecholamine responsiveness. From a clinical perspective, these data suggest that genetic polymorphism in β-ARs may account for differences in the clinical outcome of patients with chronic heart failure. In addition to these changes, some studies have shown abnormalities in *G*-protein expression. No changes in G_s-subunit were detected, while the G_i-subunit expression was found to be increased [76]. Furthermore, mRNAs for AC type V and VI tend to be reduced in the failing heart [77]. Thus, in the context of diseased heart, all these changes act to impair the signal transduction mechanisms via β_1 and β_2-ARs (Table 16.1).

16.5 Role of AC in Generation of cAMP and Regulation of PKA Activity

AC is a transmembrane protein that converts ATP to cAMP in response to stimulation of different GPCR, including β-ARs by various agonists. The mammalian AC gene family consists of 9 members, all of which are activated by $G\alpha_s$-proteins, but exhibit different patterns of regulation by other cofactors such as $G\alpha_i$- and $G\beta\gamma$-subunits, calcium, protein kinases (protein kinase C, calmodulin kinase), and phosphatases [78, 79]. Each isoform has a distinct tissue distribution, and biological as well as pharmacological properties. Types V and VI are designated as the cardiac subclasses of AC; both AC type V and VI mRNA are equally present at birth however; the AC V mRNA becomes predominant in the adult heart [80]. During heart failure, the levels of AC VI decrease while the content of AC V remains constant [77]. They have a comparable structural homology and patterns of regulation by cofactors [80, 81]. Both these AC isoforms are activated by the non-selective β-AR agonist, isoproterenol, but may have specialized functions in cardiomyocytes. The AC activity has been reported to be inhibited by submicromolar concentrations of calcium (less than 1 μM) [82]. The interaction between calcium and AC seems critical; indeed, co-localization of AC, and subunits of the L-type calcium channels along T tubules supports this hypothesis [83]. In addition, both AC isoforms can be phosphorylated by PKA resulting in a

desensitization and inhibition of AC activity [84]. Likewise, PKCα and PKCε isoforms can directly phosphorylate AC V, although PKCα is a less potent activator. PKCε activates AC V in a calcium-independent manner, whereas the PKCα requires calcium, indicating that AC V can be activated by distinct PKC isozymes under certain calcium-regulated conditions (physiological versus pathological levels of intracellular calcium). In addition, it has been suggested that cAMP production through activation of AC V is regulated by hormones and growth factors activating phosphatidyl-inositol-3,4,5 triphosphate which in turn activates PKCε [85].

During normal oncogenic development, there is a reciprocal change in the steady-state levels of mRNAs encoding type V and VI AC isoforms, while the expression of AC V increases and AC VI decreases with age [80, 86]. In the failing heart, the transcriptional level of AC V was found to be unchanged whereas the AC VI was decreased in various animal models of heart failure [77, 87, 88]. Since it is known that the proportion of β_1- and β_2-ARs is changed in the aging and diseased heart, it is possible that the abundance of AC isoforms may limit β-AR signaling in these states. In accordance, it has been suggested that these two AC isoforms may exert cardio protection through different mechanisms. In fact, overexpression of AC VI and depression of AC V are considered to be cardioprotective [89]. Cardiac-specific overexpression of AC VI has been found not to influence the basal cardiac activity, but does increase the response to β-AR stimulation [90]. In addition, enhanced survival after myocardial infarction was seen in mice with cardiac-specific overexpression of AC VI [91]. The predominant mechanism underlying this protection has been suggested to be linked with improved calcium handling as a consequence of increased phospholamban phosphorylation, reduced NCX1 and increased SR calcium content [92]. In contrast, AC VI deletion leads to impaired cardiac cAMP generation and calcium handling resulting in depressed left ventricle (LV) function (Fig. 16.3). The phenotype of AC VI knockout mice is somewhat difficult to interpret as the cardiac protein content of AC V was also found to be reduced, probably due to a post-translational mechanism. In these mice, basal contractile function and cAMP-levels were normal and β_1-AR-induced contractile function was only mildly reduced. In addition, phosphorylation of phospholamban was also reduced and the steady states SR Ca^{2+}-uptake and Ca^{2+}-storage were impaired [93]. It is pointed out that results regarding cardioprotective effects of AC VI overexpression are still not clear. Chronic aortic banding in mice with AC VI overexpression leads to impaired LV function, which is not consistent with the findings of Takahashi et al. [91] suggesting that distinct mechanisms are involved in the development of cardiac stress (pressure overload versus ischemia).

The AC V overexpression and deletion have been shown to produce opposite effects. In fact, the AC V overexpression leads to an increase in basal heart rate, an elevated LV ejection fraction and the development of cardiomyopathy with age. However, under conditions of chronic pressure overload or catecholamine stimulation, decreased LV function, increased LV hypertrophy, apoptosis and fibrosis as well as the development of pulmonary congestion and heart failure were

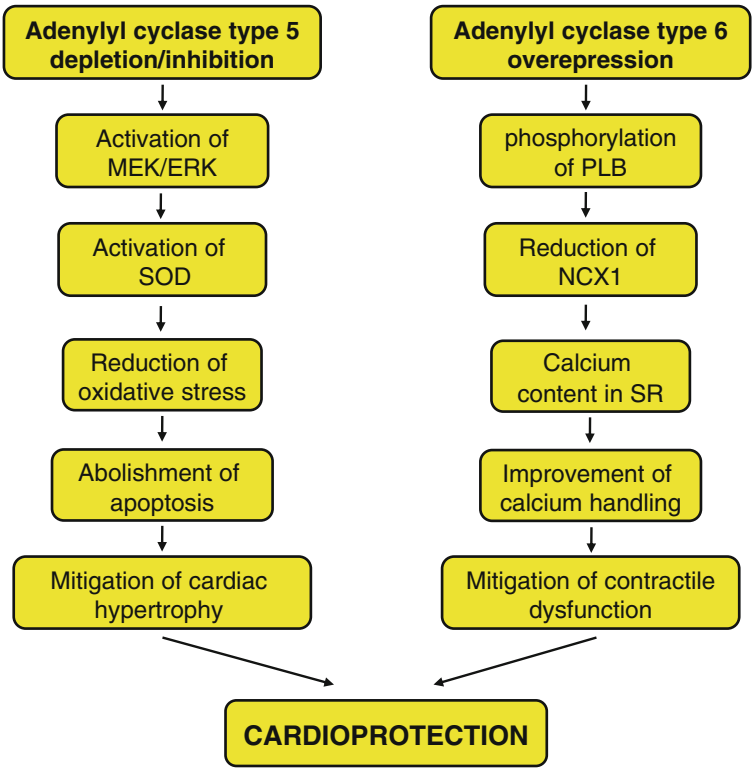

Fig. 16.3 Cardiac isoforms of adenylyl cyclase and their role in the protection of the heart against the development of cardiac hypertrophy and progression of heart failure. MEK/Erk, mitogen-activated protein kinase/extracellular-signal-regulated kinase; *SOD* superoxide dismutase 2; *PLB* phospholamban; *NCX*1 sodium/calcium exchanger 1; *SR* sarcoplasmic reticulum

documented [94, 95]. On the other hand, AC V knockout mice appear to be resistant against chronic pressure overload; these were found to have a more effective physiological response, desensitization to chronic isoproterenol infusion and a lower number of apoptotic cardiomyocytes after chronic stimulation [96]. Since abolishment of desensitization to chronic catecholamine stress is a major defence mechanism against transition of cardiac hypertrophy to heart failure, it seems that AC V is a crucial regulator of cardiac damage and induction of heart failure. In contrast, it seems that AC V does not take part in force-regulation and cAMP-production under physiological conditions [97].

It has been shown that in AC V knockout mice, despite the total protein levels of all AC isoforms in the heart and the maximal cAMP being reduced by about 50 %, both basal cardiac contractile function and heart rate were unchanged or even increased [96, 98, 99]. These interesting findings have suggested that AC V is the major G_i-inhibitable AC isoform in the heart and its absence is associated with the reduction of parasympathetic inhibition [100]. Of note, these genetically engineered

AC V knockout mice exhibit increased lifespan; they live a third longer than wild type [101]. Furthermore, these animals are resistant against aging-induced cardiomyopathy, and develop reduced cardiac hypertrophy, fibrosis, and apoptosis. In addition, AC V knockout mice have increased exercise capacity. A potential link associated with longevity of AC V knockout mice seems to be caloric restriction. Indeed, these mice and calorie restricted animals exhibit similarity in metabolism. Both these groups weigh less than their control littermates; the calorie restricted mice weigh less because of restricted food intake, however the AC V knockout mice weigh less despite increased food intake. In addition, a decrease in glycogen and blood glucose levels was observed in both groups [102, 103].

Another mechanism responsible for longevity of AC V knockout mice may be linked to attenuation of oxidative stress. In these mice, an up-regulation of manganese-superoxide dismutase (MnSOD) was detected [104]. This has been suggested to occur due to activation of the MEK/ERK signaling pathway. In addition to MEK/ERK-mediated activation of SOD resulting in decreased oxidative stress, this pathway also results in an increase in cell survival and therefore longevity (Fig. 16.3). Interestingly, bigenic mice with AC V knock out and β_2-AR overexpression do not develop the typical cardiomyopathy as is commonly observed in β_2 AR over-expressing mice [103]. In line with studies employing knockout AC V mice, a pharmacological inhibitor PMC-6, which was developed as a selective antagonist, was found to abolish β-AR-induced apoptosis. It is noteworthy that PMC-6 inhibited isoproterenol-induced cAMP accumulation only at concentrations higher than 100 nM, which is a concentration higher than that required for an maximal inotropic effect [105]. This is in agreement with an earlier report that showed the maximal force-generating effects of catecholamines in the heart occur at concentrations that are much lower than those required for maximal cAMP-increasing effects, indicating the existence of a large reserve of catecholamine-inducible cAMP [106].

16.6 Possible Benefits of Inhibiting Components of the β-AR Complex

Several groups of drugs have been designed to increase intracellular cAMP and consequently enhance the impaired myocardial contractility of the hypertrophied/failing heart. However, many of these strategies, such as cardiac directed expression of β-ARs, the stimulatory GTP-binding protein and PKA, which induce positive inotropic effects, have failed. In fact, they have resulted in LV chamber dilatation, cardiac fibrosis and heart failure [28, 107, 108]. Hence, other mechanisms related to β-AR complex have been investigated as potential targets for effective and safer pharmacological interventions. From the aforementioned discussion, it can been postulated that benefits in the treatment of cardiac hypertrophy/heart failure could be achieved by selective blockade of the β_1-ARs, inhibition of CaMKIIδC, and modulation of AC. Selective blockade of β_1-ARs maintains a β_2-mediated anti-apoptotic effect, while inhibition of CaMKIIδC directly prevents

activation of hypertrophic phenotype and apoptosis. With respect to AC, it has been suggested that selective AC VI agonists and selective AC V inhibitors could emerge as modulators of cardiac hypertrophy and heart failure. Although specific AC V inhibitors have been developed, their translation to the clinic is currently restricted due to high IC50, and low selectivity for isoform V [109, 110]. Moreover, although these inhibitors could exert similar effects as β-AR blockers the occurrence of side effects such as broncho- and vasoconstriction might limit their use since AC V and AC VI are also expressed within the bronchi and vessels [87].

16.7 Conclusions

Although a remarkable effort has been undertaken to describe pathomechanisms of cardiac hypertrophy, as well as identification of the signal transduction pathways involved, there are still many issues which need to be investigated. The involvement of β-ARs in the development of cardiac hypertrophy has been underestimated for many years, however it has become evident that they play a crucial role in the regulation of cardiac growth and apoptosis and thus account for, at least partially, decompensation of this pathological state resulting in heart failure. Molecular studies have revealed that alterations in the β-AR expression, desensitization, relocalization as well as alterations in coupling to G-proteins can underlie some phenotypes of cardiac dysfunction induced by the hypertrophic process. In addition, a different role of cardiac AC isoforms in the regulation of heart function has been recently highlighted to demonstrate that sustained stimulation of β-ARs under pathological conditions must be evaluated as a complex and not only as individual components of the signaling pathway. These findings will continue to encourage and stimulate the development of novel pharmacological agents designed to target specific proteins/isoforms.

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