Chapter 1
β-Adrenergic Receptor Signaling in Heart Failure

Grace Jung Ah Lee, Lin Yan, Dorothy E. Vatner, and Stephen F. Vatner

Abstract Acute activation of the sympathetic system and resultant β-adrenergic receptor (β-AR) signaling are required to maintain homeostasis, providing inotropic support in times of need, as in "fight or flight" or response to any stress, such as cardiac dysfunction and heart failure. For most of the twentieth century, it was reasoned that sympathetic stimulation of β-ARs through administration of naturally occurring catecholamines or synthetic sympathomimetic amines could provide inotropic support and should be used in heart failure therapy. However, in heart failure, sympathetic drive to the heart is excessively increased, and chronic sympathetic stimulation is deleterious, since it increases $M\dot{V}O_2$, which cannot be met by appropriate increases in coronary blood flow, thereby creating subendocardial ischemia and intensifying the cardiac dysfunction. Furthermore, continued stimulation of the β-ARs also becomes problematic because it can activate multiple cellular processes including those involved in pathological remodeling seen in the development of cardiomyopathy. However, this reasoning took a diametrically opposite turn in the latter twentieth century when the adverse effects of chronic β-AR stimulation became apparent from experimental studies in transgenic mice with cardiac-specific overexpression of $G_{s\alpha}$ and β-ARs and also from clinical studies with poor outcomes for patients on chronic sympathomimetic amine therapy. At this time it was also found that internal compensatory physiological processes countering continued β-AR stimulation in the heart were cleverer than physicians. As a protective response, β-AR desensitize, which reduces the effectiveness of β-AR stimulation and the consequent increases in myocardial oxygen demands. Taken together, these factors were fundamental to the change in course from β-AR stimulation to β-AR blockade in the treatment of heart failure.

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B.I. Jugdutt and N.S. Dhalla (eds.), *Cardiac Remodeling: Molecular Mechanisms*, Advances in Biochemistry in Health and Disease 5, DOI 10.1007/978-1-4614-5930-9_1,

Keywords β-Adrenergic receptor • Inotropic agonist • β-Adrenergic receptor blockers • Desensitization

1.1 Introduction

β-Adrenergic receptor (β-AR) signaling is central to all aspects of the pathophysiology of heart failure. The sympathetic nervous system including the neurohormones, epinephrine, and norepinephrine is rapidly called into action by any stress, such as cardiac dysfunction and heart failure. For most of the twentieth century, it was reasoned that sympathetic stimulation of β-ARs through administration of naturally occurring catecholamines or synthetic sympathomimetic amines could provide inotropic support and should be used in heart failure therapy. However, this reasoning took a diametrically opposite turn in the latter twentieth century when it was realized that patients with β-AR blocker therapy fared significantly better. The goal of this chapter is to document the scientific and clinical basis for the changing paradigm of the role of β-AR signaling in heart failure. To do this, the chapter has the following sections: Sect. 1.2 (The Discovery of β-ARs), Sect. 1.3 (Regulation of Cardiac Contractility by β-ARs), Sect. 1.4 (Targeting β-ARs in the Treatment of Heart Failure: Use of β-AR Inotropic Agonists), Sect. 1.5 (Adverse Effects of Chronic β-AR Stimulation in the Treatment of Heart Failure), Sect. 1.6 (Advent of β-AR Blockade Therapy), Sect. 1.7 (Mechanisms Mediating Salutary Effects of β-AR Blockade Therapy in Heart Failure), Sect. 1.8 (Future Directions), and Sect. 1.9 (Conclusions).

1.2 The Discovery of β-ARs

Although the concept of β-ARs mediating the signaling from the sympathetic nervous system to regulate cardiac function is axiomatic today, this was not always the case. Throughout much of the twentieth century, it was erroneously believed that adrenergic signaling was primarily mediated by two classes of neurotransmitters, sympathin E (excitatory) and sympathin I (inhibitory), classified according to their physiological response [1, 2]. This was due, in part, to the use of natural adrenalin, which contained variable mixtures of epinephrine and norepinephrine with quite different agonistic activities, resulting in obscured conclusions that masked their distinct effects. In retrospect, the fallacy of their results is clear. Not only do epinephrine and norepinephrine have different effects, e.g., norepinephrine has α-vasoconstrictor activity as well as β-vasodilator and inotropic activity, whereas epinephrine does not have much α-activity, but both elicit reflex effects in vivo with the most prominent mediated by the arterial baroreflex, which modulates the direct actions of the catecholamines on arterial pressure, heart rate, and peripheral vascular resistance.

In 1906, Dale first introduced the concept of receptors in connection with the sympathetic nervous system [3]. In his studies, he observed the actions of ergot alkaloid antagonists on the effects of epinephrine and proposed there are two distinct receptor types. One type, in which epinephrine mediated excitatory responses, was antagonized by ergot alkaloids, whereas in the second type, ergots had no effect on the inhibitory effects of epinephrine. Then in 1948, a major step was taken by Ahlquist, who challenged this idea of sympathins by characterizing two AR types, α and β, based on the rank order of catecholamine potencies rather than the nature of their physiological response (contraction vs. relaxation) [2].

However, the idea of ARs existing as physical entities received much skepticism [4, 5]. Even Ahlquist noted in his later paper that ARs are hypothetical structures that hold momentary value until the exact mechanism of adrenergic signaling is deciphered [6]. However, his seminal studies persevered and in 1967, Lands et al. extended his classification scheme by introducing two β-AR subtypes, β_1 and β_2, based on their affinities for epinephrine and norepinephrine [7]. Whereas β_1-ARs in cardiac and adipose tissue have approximately equal affinity for epinephrine and norepinephrine, β_2-ARs relax bronchial and vascular smooth muscle and have greater affinity for epinephrine than for norepinephrine. Then in 1972, Carlsson et al. provided pharmacological evidence that both β_1- and β_2-ARs are present and functional in the feline heart and that β_1-AR is the predominant subtype in both the atria and the ventricles [8].

From these findings, Lefkowitz developed highly specific radioligand-binding assays that allowed selective labeling of β-ARs, which was responsible for the most significant progress in the field in the latter half of the twentieth century [9]. Using this method, he and his colleagues physically identified cardiac β-ARs for the first time in the canine heart in 1975 [9]. Moreover, the radioligand-binding technique made possible the quantification of the relative proportions of β_1- and β_2-ARs and in 1983, it was reported that human left ventricle (LV) consists of 86% β_1-AR and 14% β_2-AR [10], thus confirming and extending the work of Carlsson. In addition, the interactions of β-ARs with various agonists and antagonists were explored based on the concept that the radioligand competes for the binding site with an agonist. In 1980, it was discovered that binding of an agonist and antagonist was affected by GTP [11], and taking into account that adenylyl cyclase systems require GTP for activation [12], the ternary complex model, consisting of the adrenergic receptor coupling to GTP-binding G protein to activate adenylyl cyclase (AC), was proposed [13].

The advances in molecular biology techniques that shortly followed led to the successful cloning of the β_2-AR, the very first G protein-coupled receptor to be cloned [14]. Then by the 1990s, six α-AR subtypes (α_{1A}, α_{1B}, α_{1C} and α_{2A}, α_{2B}, α_{2C}) [15, 16] and three β-AR subtypes (β_1, β_2, β_3) [17–19] were firmly established. Moreover, insights on the physiological actions of various AR subtypes were made possible through generation of transgenic mice models with targeted disruption of ARs [20–23]. Today, we now understand that α-ARs have positive inotropic activity

(most prominent in rodents), but their primary role is regulating peripheral resistance [24], whereas β-ARs provide the strongest mechanism to regulate cardiac performance [25–28].

1.3 Regulation of Cardiac Contractility by β-ARs

In response to acute stress, the normal heart must be able to rapidly increase its output nearly fivefold to meet the higher metabolic demands [29]. This is primarily met by the sympathetic nervous system acting on the β-ARs to mediate positive chronotropic and inotropic responses in the heart. While all three β-ARs subtypes, namely β_1, β_2, and β_3, are expressed on the cardiomyocyte [9, 30], β_1-AR is the most abundant form (70–80% of total β-AR in the normal heart) and is one of the main regulators of cardiac performance, with β_2-AR being secondary, but also having powerful vasodilator properties in vessels [31]. The β_3-AR, however, is only minimally expressed, and its role in the heart remains controversial. Initially, the β_3-ARs were implicated to have a role in fat metabolism [32], but various studies suggest they have cardiac roles as well. For instance, in vivo stimulation of β_3-AR induces inotropic responses in rodents; however, no effect was observed in primates, suggesting its physiological response may be species specific [33]. In addition, these reports do not correspond to the findings of ex vivo studies, which showed negative inotropic effects of β_3-AR stimulation in the ventricular tissue of mice and humans potentially through the inhibitory G protein (G_i)–nitric oxide synthase (NOS) pathway [34, 35]. The apparent discrepancy between in vivo and ex vivo effects of β_3-AR signaling may depend on the type of agonist used as well as their differential effects under in vivo and ex vivo conditions. Interestingly, however, a recent study by Niu et al. demonstrated in vivo that β_3-AR has cardioprotective roles by inducing negative inotropic effects and maintaining NO and reactive oxygen species balance in the setting of catecholamine overstimulation in the failing heart [36]. Whether this effect is also observed in humans needs to be elucidated.

β_1- and β_2-ARs are the principal stimulatory G protein (G_s)-coupled receptors that drive heart rate and enhance myocardial contractility [25–28]. Upon amine binding, both β_1- and β_2-ARs activate adenylyl cyclase (AC) to increase the level of cyclic AMP (cAMP) (Fig. 1.1) [37]. The latter targets protein kinase A (PKA), which phosphorylates various proteins involved in excitation–contraction coupling. These include, but are not limited to L-type calcium channels (LTCC) [38], ryanodine receptors (RyR2) [39], phospholamban (PLB) [40, 41], and troponin I (TnI) [41], which together coordinate stronger contractions and hastened relaxation of the cardiac muscle. For instance, phosphorylation of LTCC increases the Ca^{2+} influx [42], which stimulates RyR2 to release Ca^{2+} from the sarcoplasmic reticulum (SR). The Ca^{2+}-induced Ca^{2+} release process is further enhanced by phosphorylation of RyR2 [43], thereby increasing its sensitivity to cytosolic [Ca^{2+}] and resulting in greater SR Ca^{2+} unloading necessary for stronger pumping of the myocardium [44]. The following relaxation phase is also accelerated through phosphorylation of PLB

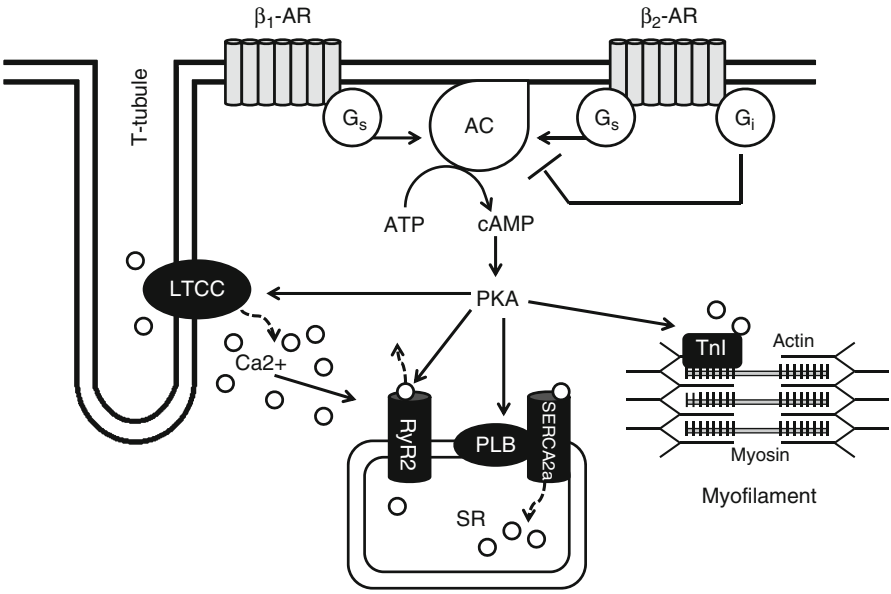

Fig. 1.1 Enhancement of cardiac contractility through β-AR signaling. Upon agonist binding, β_1- and β_2-ARs activate the stimulatory G protein (G_s)-adenylyl cyclase (AC)-protein kinase A (PKA) pathway to phosphorylate multiple calcium handling and myofilament proteins, including L-Type calcium channel (LTCC), ryanodine receptor (RyR2), phospholamban (PLB), and troponin I (TnI). Activation of these proteins by β-ARs enhances their function, resulting in stronger contractions as well as hastened relaxation. For instance, the Ca^{2+}-induced Ca^{2+} release process is further enhanced by phosphorylation of RyR2 by PKA, resulting in greater sarcoplasmic reticulum (SR) Ca^{2+} unloading during systole. The relaxation phase is also hastened by phosphorylation of PLB and TnI, resulting in faster reuptake of Ca^{2+} by the SR and faster relaxation of the myofilaments by preventing actin–myosin interaction. Interestingly, the β_2-AR also couples to inhibitory G protein (G_i), which could negate the effects of G_s–AC–PKA signaling, resulting in only modest inotropic response compared to β_1-AR

at Ser16 by PKA, which allows rapid sequestering of Ca^{2+} by relieving its inhibition of the SR Ca-ATPase (SERCA2a) pump [40, 45, 46], as well as phosphorylation of TnI, which decreases the sensitivity of myofilaments to Ca^{2+} [47–49], resulting in muscle relaxation.

Interestingly, studies from animal models reported that β_2-AR also couple to G_i, which could negate the effects of G_s–AC–PKA signaling, resulting in attenuation of enhanced contractility and hastened relaxation [50, 51]. For instance, (−)-noradrenaline hastened relaxation through β_1-ARs, but not with (−)-adrenaline through β_2-ARs in the feline ventricle [52], which was supported by the finding that β_2-ARs have modest inotropic effects compared to β_1-AR independent of receptor density in the rat heart [53, 54]. However, whether β_2-ARs couple to G_i in humans remains controversial. Kilts et al. demonstrated that β_2-ARs also couple to G_i in the human atria [55], whereas a later study by Molenaar et al. showed evidence that is inconsistent with the dual coupling feature of β_2-AR [56].

1.4 Targeting β-ARs in the Treatment of Heart Failure: Use of β-AR Inotropic Agonists

While β-AR blockers are currently widely employed in the treatment of heart failure, much of the therapeutic approaches in the twentieth century were based on the concept of increasing myocardial contractility through inotropic agents. The reasoning was simple: (1) The failing heart has poor contractility, (2) catecholamines are the most potent stimulators of myocardial contractility, and (3) catecholamines would be useful to increase contractility in patients with heart failure (quod erat demonstrandum). What was not known was either the concept of desensitization of β-AR [57–59] or the fact that there is an oxygen cost of increasing heart rate, LV wall stress, and myocardial contractility, i.e., the major determinants of $\dot{M}VO_2$, which, in turn, increase the requirement for coronary blood flow. The increased demand is easily met in normal hearts and coronary circulation, but not so in the setting of either hypertrophy or heart disease characterized by limited subendocardial coronary reserve [60, 61]. Under these conditions, the imbalance between coronary blood flow supply and myocardial oxygen demands results in myocardial ischemia, which exacerbates cardiac dysfunction.

Not recognizing these concepts, catecholamines and synthetic sympathomimetic amines were routinely administered to patients with heart failure; at first, norepinephrine or epinephrine was given [62], and then in the 1960s, isoproterenol (ISO), a nonselective β_1- and β_2-AR agonist with potent inotropic and chronotropic properties, was used in the treatment of heart failure [63]. Many patients who received this treatment developed fatal arrhythmias [63], and those with coronary artery disease had increased myocardial ischemia [64]. In an experimental correlate of the clinical situation, Hittinger et al. showed that isoproterenol infusion in conscious dogs with LV hypertrophy and failure further impaired both systolic and diastolic function [65]. The increased stress of inotropy as well as chronotropy under limited coronary flow reserve resulted in subendocardial hypoperfusion, which exacerbated the myocardial dysfunction.

In an effort to find a selective agent that could augment the inotropic state of the myocardium without affecting the chronotropic state, dopamine, a naturally occurring, nonselective α- and β-AR agonist, was used. While dopamine had less chronotropic effect compared to isoproterenol, the increase in cardiac index was still associated with elevated heart rate, resulting in ventricular tachyarrhythmias [66, 67]. Furthermore, the inotropic effect of dopamine was mediated in part by the release of endogenous noradrenaline [68–70]. In severe heart failure, where endogenous noradrenaline levels are low, the degree to which dopamine could increase cardiac output was often minimal and unpredictable [71, 72]. Moreover, infusion at high doses leads to stimulation of both α- and β-ARs with a predominant α-AR effect, resulting in excessive vasoconstriction [73, 74]. To minimize the effects on peripheral vasculature and heart rate, while retaining positive inotropic effects, dobutamine, a synthetic derivative of dopamine, was developed. Vatner et al. investigated the actions of dobutamine in healthy conscious dogs and found that it

exerted potent inotropic effects with insignificant changes in heart rate [75]. However, the lack of increase in heart rate and effects on peripheral vascular resistance was due to mixed α- and β-adrenergic stimulating properties of the drug, coupled with counteracting influences induced by activation of autonomic reflexes. Accordingly, when autonomic reflexes were blocked or when one arm of the AR system (either α or β) was blocked, effects on heart rate and peripheral vascular resistance were identified. Furthermore, considering that many inotropic agents also have vasodilating effects, administration of nitroprusside, a vasodilating agent, was also explored. In the 1970s, there were various reports showing the promising effects of nitroprusside in improving heart function in the setting of heart failure [76–78]. A later study compared the effects of chronic infusion of dobutamine with those of nitroprusside in end-stage heart failure patients and showed that patients with nitroprusside treatment had significantly higher event-free survival rate than patients with dobutamine treatment [79]. From these findings, it was proposed that peripheral vasodilator therapy with nitroprusside was superior to dobutamine. However, the combined effects of vasodilators and inotropes resulted in a higher mortality rate than vasodilator therapy alone [80]. In the early 1980s, moderate success was observed with the β_1-AR selective inotropic agent, prenalterol. Although in an acute setting, it improved hemodynamics [81, 82], chronic administration showed detrimental effects [83]. Then in the 1990s, clinical trials with β_2-AR agonist, dopexamine, initially showed some clinical benefit over the placebo [84, 85]. However, the beneficial effects were due to the vasodilatory and cardiac unloading effects of this agonist.

In an attempt to improve cardiac function, β-AR downstream targets were also explored. Milrinone, a phosphodiesterase (PDE) inhibitor, was first approved for intravenous use in the late 1980s due to its effects on increasing cAMP half-life and subsequently increasing intracellular Ca^{2+} concentration and improving cardiac contractility. In addition, because increased cAMP levels also results in arterial and venous dilation, it may lead to hypotension. In clinical trials, milrinone had no clear benefit over placebo [86]. Then in 1986, enoximone, another PDE inhibitor, was studied. A year later, it was found to be superior to both dobutamine and nitroprusside in the management of heart failure [87]. However, in 1994, it was found that enoximone administration in patients with end-stage heart failure resulted in increased mortality [88]. Then in a larger phase III trial in 2009, ESSENTIAL-I and ESSENTIAL-II, enoximone treatment did not show any benefit over the placebo, which led to the termination of enoximone development [89].

1.5 Adverse Effects of Chronic β-AR Stimulation in the Treatment of Heart Failure

Acute activation of the sympathetic system is required to maintain homeostasis, providing inotropic support in times of need, such as in “fight or flight” situations. However, in heart failure, sympathetic drive to the heart is excessively increased,

and as discussed in the last section, the chronic sympathetic stimulation is deleterious, since it increases $\dot{M}VO_2$, which cannot be met by appropriate increases in coronary blood flow. This results in subendocardial ischemia, which intensifies the cardiac dysfunction. Furthermore, continued stimulation of the β-ARs also becomes problematic because it can activate multiple cellular processes including those involved in pathological remodeling seen in the development of cardiomyopathy. Therefore, continued β-AR stimulation as occurs in heart failure induces β-AR desensitization [57–59], which is protective since it reduces the effectiveness of β-AR stimulation and the consequent increases in myocardial oxygen demands. The realization of these concepts was fundamental to the paradigm shift from β-AR stimulation to β-AR blockade in the treatment of heart failure. The background documenting this concept follows in both experimental and clinical settings.

1.5.1 Experimental Evidence

It was a considerable time before the adverse outcomes of chronic β-AR stimulation were demonstrated both in vitro and in vivo in numerous animal studies. Initially, it was thought that β-AR overexpression could be a novel therapy for heart failure. This concept was supported by studies that showed enhanced myocardial function in transgenic mice with up to 60-fold overexpression of β_2-ARs [90] without evidence of cardiac pathology [91]. Furthermore, transgene therapy with adenovirus coding the β_2-AR transgene significantly improved cardiac function in the failing rabbit hearts [92].

However, the error of this concept was realized when the chronic effects of β-AR stimulation were observed. The novel feature of the following studies was the continued monitoring of the mice as they aged. Iwase et al. reported that transgenic mice with cardiac-specific overexpression of $G_{s\alpha}$ ($G_{s\alpha}$ TG) have increased responsiveness to β-AR stimulation by ISO compared to WT mice as young adults, as evident by significantly higher left ventricular ejection fraction (LVEF) (Fig. 1.2a) [93]. However, upon aging, their function deteriorates, resulting in LV dilation, higher incidence of arrhythmias, and depression of LVEF (Fig. 1.2a) [93–95]. Furthermore, histological examination of these hearts revealed a picture of cardiomyopathy, including marked increase in hypertrophy, myocyte necrosis, and fibrosis (Fig. 1.2b) [93]. Confirming these findings, Engelhardt et al. showed that transgenic mice with cardiac-specific overexpression of β_1-AR (β_1-AR TG) have hyperfunction at a young age, but their function progressively worsens as the mice age [96], which was also further confirmed by the work of Peter et al. (Fig. 1.3a) [97]. Furthermore, premature mortality by 12 months of age was significantly higher in β_1-AR TG mice compared to WT mice (Fig. 1.3b) [98]. In support of these findings, in vitro studies in rat ventricular myocytes showed significant increases in apoptosis upon β_1-AR stimulation [100], and hypertrophy from ISO administration was abolished by a β_1-AR antagonist [101]. Furthermore, the concept of the beneficial effects of chronic β_2-AR overexpression was finally put to rest when Du et al. showed that

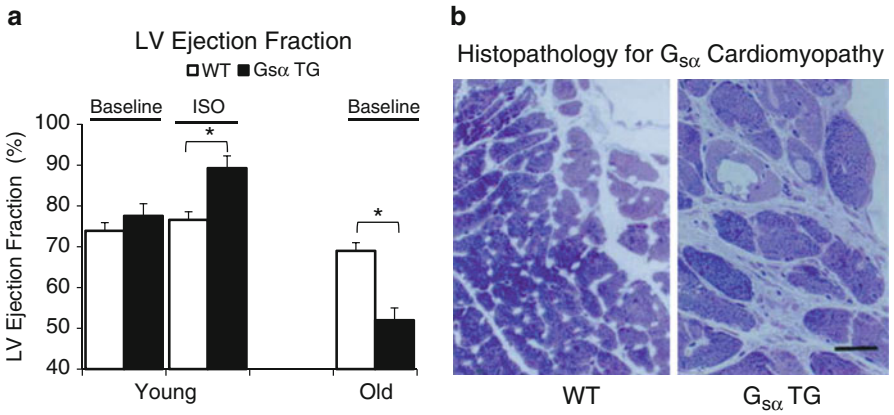

Fig. 1.2 Overexpression of $G_{s\alpha}$ in the heart induces cardiomyopathy. (**a**) $G_{s\alpha}$ TG mice have higher responsiveness to isoproterenol (ISO) compared to WT mice as young adults, but experience progressive deterioration of left ventricular ejection fraction (LVEF) with age (*$p<0.05$) [93, 94]. (**b**) LV subendocardium of 19-month-old WT mice and 15-month-old $G_{s\alpha}$ TG mice stained with toluidine blue. Old $G_{s\alpha}$ TG mice have marked cellular hypertrophy and fibrosis compared to WT mice. Bar = 25 μm [93]. Figures adapted with permission from Lippincott Williams & Wilkins and Journal of Clinical Investigation

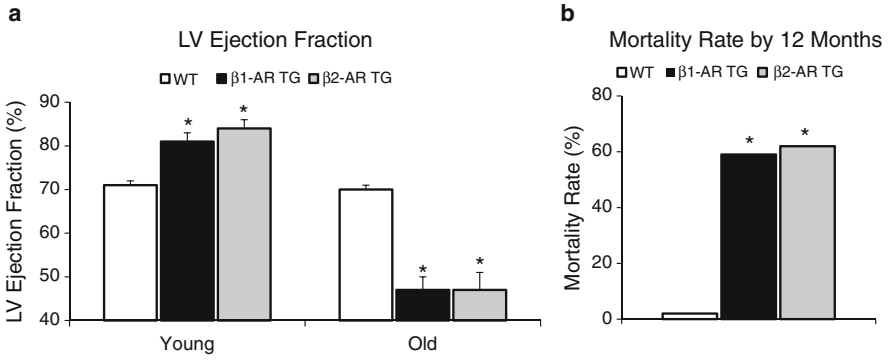

Fig. 1.3 Cardiac overexpression of β_1-AR or β_2-AR decreases heart function and increases mortality with age. (**a**) β_1- and β_2-AR TG animals have enhanced left ventricular ejection fraction (LVEF) as young adults, but their cardiac function significantly worsens as the animals age compared to the age-matched WT mice. (*$p<0.05$ vs. WT) [97]. (**b**) The mortality rate of β_1- and β_2-AR TG animals by 12 months of age is significantly higher compared to WT animals (*$p<0.05$ vs. WT) [98, 99]. Figures adapted with permission from Journal of Clinical Investigation, Lippincott Williams & Wilkins, and Oxford University Press

β_2-AR TG mice also develop progressive cardiomyopathy with increasing age, as reflected by deterioration of myocardial function [99], which was also confirmed by studies of Peter et al. (Fig. 1.3a) [97]. Similar to β_1-AR TG mice, β_2-AR TG mice also have significantly higher mortality by 12 months of age compared to the WT mice (Fig. 1.3b) [99].

Chronic stimulation of the β-AR pharmacologically with high doses of ISO also results in the rapid development of cardiomyopathy. Similar to transgenic mice models of cardiomyopathy, several studies in rodent models have reported marked increases in cellular necrosis and fibrosis [102–105] and impaired LV function [106, 107] upon administration of high doses of ISO, resulting in heart failure and increased mortality. In support of this, the effects of chronic catecholamine stress are exacerbated in mice with transgenic overexpression of components in the β-AR signaling pathway, such as AC5, one of the two major AC isoforms in the heart. It was shown that mice with cardiac-specific overexpression of AC5 (AC5 TG) have higher mortality, depressed LVEF, as well as increased fibrosis and apoptosis compared to the WT mice under chronic catecholamine stress [108].

It is now recognized that the development of cardiomyopathy from chronic β-AR stimulation is mediated by several signaling pathways (Fig. 1.4). One of these is the Ca^{2+} handling pathway. In animal models as well as heart failure patients, prolonged Ca^{2+} transients have been described [109, 110], and in humans, it was shown that this effect was due, in part, to the impairment of reuptake of Ca^{2+} into the SR during diastole [111, 112]. In fact, it was shown that in the failing heart, PLB is hypophosphorylated by PKA [113], resulting in delayed sequestering of Ca^{2+} by the SR and increased cytosolic Ca^{2+} during diastole, which is further exacerbated by enhanced activity of LTCC [114]. Engelhardt et al. showed that adverse effects of cardiac-specific overexpression of β_1-AR could be rescued by ablation of PLB [98]. The β_1-AR TG x $PLB^{-/-}$ bigenic mice had significantly lower diastolic calcium levels and upregulation of SERCA compared to β_1-AR TG mice. Moreover, the bigenic mice had significant improvement in mortality and cardiac function as well as reduction in hypertrophy and fibrosis. The cardiotoxic effects of increased intracellular Ca^{2+} could be attributed to activation of calcineurin [115] and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) pathway [116], which could induce hypertrophy. However, it may also be due to the activation of the necrosis pathway. While Ca^{2+} is not the main regulator of the necrosis pathway, previous studies have suggested that increases in intracellular Ca^{2+} induced by β-AR stimulation by ISO could induce necrosis [117, 118].

Other distal signaling pathways that have been implicated involve several kinases, which mediate hypertrophy and cardiomyopathy. For example, inhibition of proto-oncogene serine/threonine-protein kinase (Raf-1), which activates MAPK/ERK kinase (MEK) and subsequently extracellular signal-regulated kinase (ERK), may contribute to the development of cardiomyopathy. Yan et al. showed that mice with AC5 disruption (AC5KO) were protected against aging cardiomyopathy, and the mechanism behind this protection was proposed to be enhanced pro-survival Raf-1/MEK/ERK signaling [119]. This is also supported by the finding that ERK activation could prevent myocardial necrosis and apoptosis [120–122] and ERK inhibition under ischemic insult results in increased size of myocardial infarction in vivo [120] as well as increased apoptosis in vitro [123]. Therefore, it may be possible that inhibition of this pro-survival pathway may contribute to the development of cardiomyopathy. Another pathway that contributes to the development of cardiomyopathy involves activation of proapoptotic protein p38α mitogen-activated protein kinase (p38α MAPK), which was shown to be elevated in $G_{s\alpha}$ TG cardiomyopathy

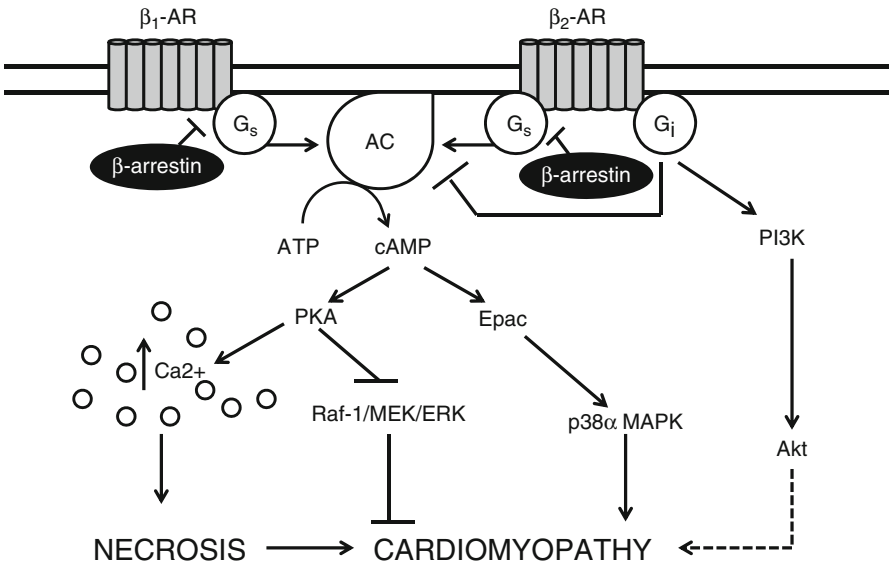

Fig. 1.4 β-AR signaling pathways involved in the development of cardiomyopathy. Chronic stimulation of β_1- and β_2-ARs drives cAMP levels via G_s-AC signaling to activate protein kinase A (PKA) and Epac. PKA induces enhanced activity of Ca^{2+} handling proteins, such as LTCC and RyR2, resulting in significant increases in cytosolic Ca^{2+} levels, which contributes to necrosis and subsequent development of cardiomyopathy. PKA could also inhibit the pro-survival proto-oncogene serine/threonine-protein kinase (Raf-1), mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK), and extracellular signal-regulated kinase (ERK) pathway, thereby relieving this protective mechanism. Similarly, Epac activates p38α mitogen-activated protein kinase (p38α MAPK) to induce cardiomyopathy. The β_2-AR also couples to G_i to activate the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (Akt) pathway. While acute stimulation of Akt may be beneficial, chronic stimulation of this protein may have adverse effects. As a protective measure, β-arrestins are recruited to decouple the receptor from downstream signaling, thereby desensitizing the β-AR

model [124]. Peter et al. reported that inhibition of p38α MAPK rescues the cardiomyopathy induced by β_2-AR [97]. While p38α MAPK disruption did not prevent β_1-AR-induced cardiomyopathy, there was upregulation of another proapoptotic protein, mammalian sterile 20-like kinase 1 (Mst-1) in the hearts of β_1-AR TG mice [97], suggesting that Mst-1 may play a role in the development of cardiomyopathy that is more associated with the β_1-AR. The last pathway, which involves phosphatidylinositol 3-kinase (PI3K)–protein kinase B (Akt) signaling, is a matter of debate. Various studies have shown that Akt mediates hypertrophy [125–127], but could also enhance cardiac contractility [128, 129], suggesting it has beneficial roles in the heart. Kim et al. showed that mice with cardiac-specific overexpression of active Akt had increased L-type Ca^{2+} density, as well as increased expression of SERCA2a protein, which resulted in enhanced myocardial contractility [128]. Furthermore, it has been well documented that Akt can protect the heart from apoptosis in vitro [130, 131]

and in vivo [132]. Okumura et al. reported that mice with AC5 disruption (AC5KO) had significantly lower apoptosis compared to WT mice after ISO administration, which was associated with marked increase in active Akt [133]. However, other studies have highlighted the deleterious effects of Akt signaling. Matsui et al. reported that chronic Akt expression in mice results in a vast array of phenotypes, including moderate cardiac hypertrophy with preserved function to significant cardiac dilation as well as sudden death [134]. In a later study, Nagoshi et al. reported that transgenic mice with cardiac-specific Akt expression had significantly larger infarcts without any restoration of function under ischemia/reperfusion injury [135]. Furthermore, it was shown that activation of Akt results in a negative feedback mechanism to inhibit PI3K, concluding that in the heart, PI3K-dependent but Akt-independent pathways are required for full cardioprotection. Given these findings, the possibility of Akt contributing to the development of cardiomyopathy cannot be fully dismissed.

One of the key series of studies instrumental in changing the concept of β-AR stimulation to β-AR blockade in heart failure therapy involved the mouse models with cardiac-specific overexpression of either $G_{s\alpha}$ or β-AR. As noted earlier, these mice responded to increased sympathetic stimulation with enhanced levels of heart rate and contractility (Fig. 1.2a) [93]. This was not a transient process, as occurs with intravenous administration of catecholamines, but was a permanent fixture of their cardiac function through constant overexpression. The $G_{s\alpha}$ mice tolerated the chronically enhanced cardiac function when they were young, but developed cardiomyopathy as they aged, as reflected by cardiac dysfunction and heart failure, myocyte necrosis and apoptosis and cardiac fibrosis, as well as premature mortality from the cardiomyopathy. When these mice were put on continuous β-AR blockade therapy, they were protected from cardiomyopathy and showed improvement in LVEF as well as improvement in survival (Fig. 1.5a) [94]. These findings were not only novel at the time, but also played a significant role in the paradigm shift from β-AR agonist to β-AR blockade in the treatment of heart failure.

1.5.2 Clinical Evidence

A major deterrent to β-AR stimulation therapy was that patients who received inotropic agonists often experienced side effects, including elevated heart rate, arrhythmias, and peripheral vasoconstriction, which overshadowed any initial inotropic benefits gained. Moreover, patients with treatment experienced worsening of their condition and significantly higher mortality rates than the placebo group. For instance, ISO induced fatal arrhythmias and intensified myocardial ischemia in patients with coronary artery disease. Patients with dopamine treatment developed ventricular tachyarrhythmias. Moreover, prenalterol significantly increased mortality. In addition, while dobutamine had beneficial effects in the acute setting, chronic administration had adverse effects. In fact, the 1999 Flolan International Randomized Survival Trial proved dobutamine to increase mortality [137]. These findings were critical in the shift from beta agonists to β-AR blockers in the treatment of heart failure.

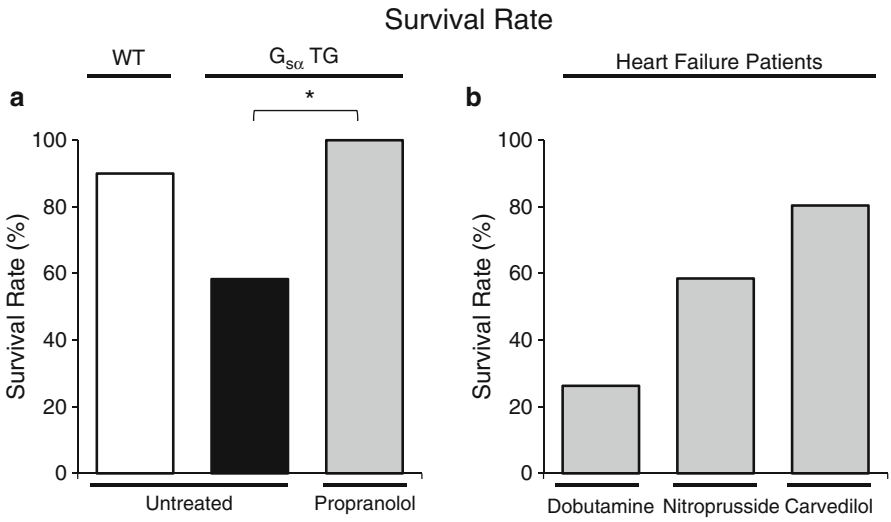

Fig. 1.5 β-AR blockade treatment increases survival rate. (**a**) Approximately 9-month-old WT and $G_{s\alpha}$ TG mice were followed for mean duration of 6.5 months with or without propranolol treatment. In the untreated group, $G_{s\alpha}$ TG mice have premature mortality, which was abolished by propranolol treatment. (*$p<0.05$ by log-rank test) [94]. (**b**) By 300 days of therapy, β-AR blocker, carvedilol, was shown to be superior in prolonging the lifespan of heart failure patients compared to both β-AR agonist (dobutamine) and vasodilator agent (nitroprusside) [79, 136] Figures moadapted with permission from Journal of Clinical Investigation and Oxford University Press

The other major clinical contribution was the concept of β-AR desensitization in patients with heart failure introduced by Bristow and his colleagues [57]. As an adaptive response to increased catecholamine levels, β-ARs desensitize through downregulation of the receptors and attenuation of downstream signaling. Bristow et al. demonstrated that isoproterenol (ISO) stimulation in failing human hearts resulted in 50–56% reduction in β-AR density, decreased AC activity, and decreased muscle contraction compared to normal hearts [57], and the half-equivalent dose of ISO was fivefold higher in the failing tissue compared to the normal tissue [138]. Dose–response study with dobutamine showed progressively lower inotropic response (dP/dt) in patients with severe heart failure compared to those with moderate heart failure [139]. The observed β-AR desensitization is mediated by two mechanisms. First, β-AR mRNA is downregulated [140, 141] potentially through degradation by A + U-rich element RNA-binding/degradation factor (AUF1) [142]. It was found that AUF1 expression is significantly elevated in individuals with heart failure, and its abundance was regulated by β-AR stimulation. In addition, AUF1 was able to interact with β-AR mRNA suggesting it may be responsible for its stability. Second, β-ARs are decoupled from downstream signaling [143–145].G protein-coupled receptor kinase 2 (GRK2), a member of the GRK family commonly known as β-AR kinase 1 (βARK1), phosphorylates agonist occupied β-AR [146] and subsequently recruits

β-arrestin, which inhibits G protein-mediated signaling (Fig. 1.4) [147]. Studies in the left ventricles of patients with dilated or ischemic cardiomyopathy showed elevated expression and activity of GRK2 [141].

Therefore, in the setting where β-ARs are already desensitized, β-AR agonist treatment will not provide much inotropic response. In fact, it will exacerbate the condition, as seen in the clinical trials of β-AR agonists. For these reasons, the concept of β-AR desensitization was important in understanding the rationale for β-AR blockers in heart failure therapy.

1.6 Advent of β-AR Blockade Therapy

β-AR blockade therapy began with the seminal studies of Sir James Black, who was awarded the Nobel Prize in 1988 [148] for developing β-AR blockers in the treatment of cardiac disease. He began his studies with the goal of reducing myocardial oxygen demands [149], and by 1962, he and Stephenson introduced a nonselective β-AR blocker, pronethalol [150]. It was reported that pronethalol had strong antiarrhythmic effects in guinea pigs and dogs [151–154] and was effective in managing angina pectoris in patients [155]. Shortly after, Black developed another nonselective β-AR blocker, propranolol, in 1965 [156]. He compared the effects of pronethalol with propranolol and showed that propranolol was superior in reducing heart rate and blocking ISO-induced hypotension without any fall in blood pressure in dogs [156].

Furthermore, unlike inotropic agonists, propranolol treatment in mice did not induce myocardial damage [157]. In fact, it was shown to be effective in reducing mortality rates and ventricular fibrillation in experimental studies of coronary artery occlusion [158, 159]. Similar effects were seen in the clinical setting, and it was reported that propranolol was effective in improving myocardial oxygenation [160] and reducing mortality in patients with myocardial infarction [161]. Furthermore, it had strong antiarrhythmic effects as well as having beneficial effects in controlling angina pectoris in heart failure patients [162–164]. The success of propranolol also led to the development of β_1-AR selective β-AR blockers including practolol, atenolol, and metoprolol. While practolol had less β-AR blocking potency than propranolol, animals studies demonstrated that practolol was effective in preventing arrhythmias and other alterations in cardiac function [165]. Furthermore, administration of atenolol or metoprolol in mice was able to prevent myocardial injury induced by epinephrine, including inflammatory cell infiltration, fibrosis, and atrophy and necrosis [166]. Then in 1975, the beneficial effects of practolol and alprenolol were illustrated clinically [167]. Six patients were treated with oral β_1-AR selective blocker practolol, and one patient received the nonselective β-AR blocker, alprenolol, for 2–12 months. After treatment, all seven patients showed improvement in ventricular function and reduction in heart size. The long-term effect of β_1-AR

selective blocker, metoprolol, was also evaluated in an international multicenter study, the metoprolol in dilated cardiomyopathy (MDC) trial [168]. Beginning from 1985, 383 patients were followed for at least 12 months. Although the beneficial effects of metoprolol were modest during the first year of treatment, the requirement for heart transplantation was dramatically decreased in the treatment group. In addition, metoprolol was well tolerated in the long term, indicated by the low withdrawal rate. In 1994, the trial on the β_1-AR selective blocker bisoprolol in the cardiac insufficiency bisoprolol study (CIBIS) was published [169] and was soon followed by the US Carvedilol (nonselective β/α_1-AR blocker) Study in 1996 [136]. Patients with carvedilol treatment had significant improvement in mortality, and the survival benefit compared to β-AR agonist, dobutamine, as well as to vasodilator agent, nitroprusside, is clear (Fig. 1.5b). The first large randomized clinical trial was the Cardiac Insufficiency Bisoprolol Study II (CIBIS-II), released in 1999, in which 2,647 heart failure patients were followed for a mean duration of 1.3 years [170–173]. The study was terminated early due to the clear benefit of bisoprolol in decreasing mortality rate (11.8% vs. 17.3% bisoprolol vs. placebo). In 2000, similar results were seen in the Metoprolol CR/XL Randomized Intervention Trial in congestive heart failure (MERIT-heart failure) [174].

1.7 Mechanisms Mediating Salutary Effects of β-AR Blockade Therapy in Heart Failure

While β-AR blockers improve cardiac function as well as hemodynamic response, the concept that β-AR blockade would be useful therapy in heart failure is counter-intuitive, with the inverse reasoning of why β-AR stimulation would be useful in heart failure, alluded to earlier. In addition, β-AR blockade has already been initiated by failing heart through desensitization and reduced β-AR density and regulation, also noted earlier. To put it simply the failing heart has an internal brain telling the physician how to design its therapy, i.e., if β-AR blockade is already initiated physiologically, then it follows that more of the same might be indicated. The counter argument is based on knowing that β-AR blockers have a profound cardiac depressant effect, which could not be tolerated in a heart already failing with marked cardiac depression. Indeed, early attempts at β-AR blockade often failed because of this. It was not until physicians recognized that by gradually and incrementally instituting β-AR blockade was it possible to achieve therapeutic levels without compounding the cardiac depression. This is combined with the fact that some of the current β-AR blockers used clinically, e.g., carvedilol, have actions other than simple β-AR blockade, such as vasodilating effects, and are as not as potent β-AR blockers as propranolol.

Empirically noting the above does not mean that all the mechanisms mediating the salutary action of β-AR blockade in heart failure are known. In fact, it remains unclear how β-AR blockers lower the risk of cardiac complications. However, it is

apparent that β-blockers offer far more than simply blocking the receptor. First of all, again with inverse reasoning to why β-AR stimulation therapy failed clinically, β-AR blockers reduce heart rate, arguably the most important regulator of $\dot{M}VO_2$. As noted above, the imbalance between myocardial oxygen supply and demand is an important mechanism resulting in myocardial dysfunction through invocation of subendocardial ischemia. Since the most important regulator of myocardial oxygen demand is heart rate followed by LV wall stress, a drug which diminishes heart rate and prevents subendocardial ischemia will then reduce LV wall stress. These hemodynamic factors alone will eventually act to prevent a further decline in cardiac function followed by gradual recovery. This also results in a "resensitizing" effect. These include upregulating β-AR density, decreasing GRK2 activity, correcting the impairment of Ca^{2+} handling proteins, and reversing downstream signaling.

1.7.1 Upregulation of β-AR Density

Reduced contractility in chronic heart failure through desensitization mechanisms is protective, as it reduces myocardial oxygen demand, and more importantly, the sudden increases that occur with stress. As noted earlier, Bristow et al. initially reported that β-AR density is reduced by 50% in failing human ventricles, and later studies have confirmed this finding. Therefore, it is conceivable that increases in cardiac performance with β-blockade result, in part, from quantitative restoration of β-AR. In the normal rat heart, chronic infusion of nonselective β-AR blocker, propranolol, was associated with significant increases in both β_1- and β_2-AR density [175]. In addition, heart failure patients with β_1-AR blocker treatment, metoprolol, also showed upregulations of cardiac β-ARs [176, 177]. The mechanism behind this action is uncertain. However, the synthesis and degradation of β-ARs seem to be regulated on the RNA level. As mentioned earlier, AUF1 is significantly increased in heart failure and was shown to degrade β-ARs and thereby reducing its density [142]. In addition, the increase of β-ARs following of β-AR blockade was seen on the protein level as well as on the mRNA level [140]. Interestingly, however, carvedilol and bucindolol, nonselective β-AR blockers with vasodilating properties through α_1-AR inhibition, did not exert β-AR upregulating effects in heart failure patients, despite being as effective in improving cardiac function [176, 178]. It may be possible that these classes of β-AR blockers have other mechanisms mediating their beneficial effects.

1.7.2 Decreasing GRK2 Activity

The increase in inotropy seen in patients with β-AR blockade may indicate that there is an enhancement of β-AR signaling from its depressed state. GRK2 is a G protein-coupled receptor kinase responsible for phosphorylating the β-ARs and

decoupling the receptor from downstream signaling via recruitment of β-arrestin. Patients with heart failure have elevated GRK2, consistent with the β-AR desensitization concept. In addition, studies in transgenic mice have shown that increase in GRK2 has negative inotropic response, which could be ameliorated by GRK2 inhibition [179]. Given this finding, it may be possible that improvement in contractile response seen in patients with β-AR blockade may be partly due to decreased GRK2 activity. In fact, studies in mice [180] and pigs [181] have shown that treatment with bisoprolol, atenolol, and carvedilol downregulate GRK. This effect was also seen in heart failure patients. Treatment with metoprolol or bisoprolol decreased GRK2 activity in the right atrium compared to patients who did not receive β-AR blockers [182, 183]. Therefore, downregulation of GRK2 may be an important mechanism by which β-AR blockers confer beneficial effects.

1.7.3 Correcting the Impairment of Ca^{2+} Handling Proteins

The decreased β-AR signaling also leads to subnormal phosphorylation of Ca^{2+} handling proteins. In fact, PLB and TnI have been shown to be hypophosphorylated in the failing hearts [113, 184, 185]. Decreased phosphorylation of PLB results in delayed SR Ca^{2+} uptake, which in part explains the relaxation deficit in heart failure patients, as well as decreased SR unloading, resulting in weaker contractions. Interestingly, however, RyR2 have been reported to be hyperphosphorylated in heart failure [39]. The result is a "leaky RyR2" that leads to higher diastolic Ca^{2+} concentrations and a decreased Ca^{2+} loading of the SR, which leads to higher propensity for arrhythmias. Given that β-AR signaling is attenuated in heart failure, it may be possible that β-AR blockade could correct these impairments through resensitizing the system, thereby restoring the phosphorylation of these proteins to normal levels. In fact, patients with carvedilol, metoprolol, or atenolol treatment had restoration of normal phosphorylation of RyR2 associated with improved cardiac muscle function [186].

1.7.4 Reversing Adverse Effects of Distal Signaling

It is also important to note that β-AR blockers could reverse the maladaptive signaling in distal mechanisms. For instance, the increase in levels of proteins involved in cellular growth and death, such as p38α MAPK, Jun N-terminal kinase (JNK), and Akt, seen in $G_{s\alpha}$ cardiomyopathy model was downregulated upon propranolol treatment [124]. In parallel to this evidence, patients who responded to either metoprolol or carvedilol had restoration of adult α-myosin heavy chain isoform and reduction of the fetal β-isoform levels [187, 188]. This effect could be due to decreased circulating catecholamine levels from β-AR blockade because re-induction of fetal genes and shift in myosin heavy chain isoform occur in parallel to the elevated levels of

catecholamines seen in heart failure patients [189]. From these findings, it is clear that β-AR blockade does not simply antagonize the receptor, but also affects multiple levels of the signaling pathway to salvage the heart.

1.8 Future Directions

The treatment of heart failure and the reduction in mortality and morbidity have all improved markedly over the past several decades; however, this disease remains a leading cause of mortality and morbidity. Accordingly, there is considerable room for improvement in therapy. Advances will occur in finding new components of the β-AR signaling pathway to inhibit that are distal to the β-AR and have less adverse depressant effects on LV function. This could be at the level of inhibiting adenylyl cyclase [190] or even more distal signaling mechanisms, e.g., Raf/MEK/ERK, p38 MAPK, and other kinases yet to be defined. It is interesting that inotropic therapy is not dead, and new agents are being devised to stimulate the failing heart, without the adverse consequences of increasing $\dot{M}VO_2$. One example is the development of cardiac myosin activators, which improve the performance of the failing heart in chronically instrumented conscious dogs and patients with heart failure [191–193]. In contrast to sympathomimetic amines, such as dobutamine or dopamine, the cardiac myosin activators do not increase $\dot{M}VO_2$ (Fig. 1.6a), most likely because they reduce heart rate and do not increase LV wall stress. The most unique aspect of their action is to increase stroke volume and cardiac output by increasing the duration of cardiac contraction, and not by increasing the rate of LV pressure development (LV dP/dt) (Fig. 1.6b, c). These drugs along with those affecting Ca^{2+} and particularly at the level of the ryanodine receptor [39] could be responsible for the next advances in heart failure therapy.

1.9 Conclusions

This chapter summarized the development of β-AR stimulating agents for the treatment of heart failure from the discovery of β-ARs to how they regulate cardiac contractility and their use for most of the twentieth century in cardiac therapy. There are very few therapeutic approaches that demonstrated a diametrically opposite turn as did the transition from β-AR agonists to antagonists in the treatment of heart failure. The reasoning for treating heart failure with β-AR agonists was simple: (1) the failing heart has poor contractility, (2) catecholamines were the most potent stimulators of myocardial contractility, and (3) catecholamines would be useful to increase contractility in patients with heart failure (quod erat demonstrandum). What was not known was either the concept of desensitization of β-AR or the fact that there is an oxygen cost of increasing heart rate, LV wall stress, and myocardial contractility, i.e., the

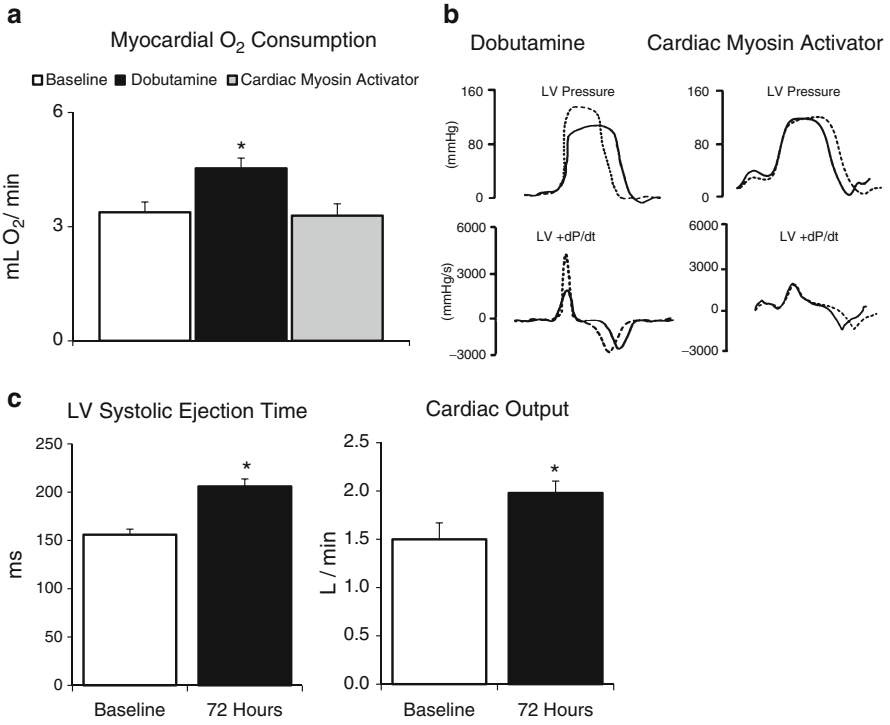

Fig. 1.6 Cardiac myosin activators improve cardiac function in hearts with systolic dysfunction. (**a**) Unlike dobutamine, cardiac myosin activators do not increase myocardial O_2 consumption (*$p<0.05$ vs. baseline) [191]. (**b**) Compared to dobutamine, cardiac myosin activators do not increase LV pressure development (solid line: before treatment, dashed line: after treatment) [191]. (**c**) 72-h infusion of cardiac myosin activator significantly increases LV systolic ejection time and cardiac output from the baseline (*$p<0.05$ vs. baseline) [191]. Figures adapted with permission from Lippincott Williams & Wilkins

major determinants of $M\dot{V}O_2$, which, in turn, increase the requirement for coronary blood flow. The increased myocardial metabolic demand is easily met in normal hearts and coronary circulations, but not so in the setting of either hypertrophy or heart disease characterized by limited subendocardial coronary reserve. Under these conditions, the imbalance between coronary blood flow supply and myocardial oxygen demands results in myocardial ischemia, which exacerbates cardiac dysfunction. All of these factors were fundamental to the change in course from β-AR stimulation to β-AR blockade in the treatment of heart failure, reinforced by poor clinical outcomes of patients on β-AR stimulation therapy. This chapter elucidates the experimental and clinical evidence delineating the mechanisms mediating increased cardiac function with β-AR agonists and conversely the deleterious effects of chronic stimulation, which can lead to heart failure, resulting in a transition for heart failure therapy from β-AR stimulation to blockade.

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