

Modulation of β -adrenergic receptor signaling in heart failure and longevity: targeting adenylyl cyclase type 5

David Ho · Lin Yan · Kousaku Iwatsubo ·
Dorothy E. Vatner · Stephen F. Vatner

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Abstract Despite remarkable advances in therapy, heart failure remains a leading cause of morbidity and mortality. Although enhanced β -adrenergic receptor stimulation is part of normal physiologic adaptation to either the increase in physiologic demand or decrease in cardiac function, chronic β -adrenergic stimulation has been associated with increased mortality and morbidity in both animal models and humans. For example, overexpression of cardiac Gs α or β -adrenergic receptors in transgenic mice results in enhanced cardiac function in young animals, but with prolonged overstimulation of this pathway, cardiomyopathy develops in these mice as they age. Similarly, chronic sympathomimetic amine therapy increases morbidity and mortality in patients with heart failure. Conversely, the use of β -blockade has proven to be of benefit and is currently part of the standard of care for heart failure. It is conceivable that interrupting distal mechanisms in the β -adrenergic receptor-G protein-adenylyl cyclase pathway may also provide targets for future therapeutic modalities for heart failure. Interestingly, there are two major isoforms of adenylyl cyclase (AC) in the heart (type 5 and type 6), which may exert opposite effects on the heart, i.e., cardiac overexpression of AC6 appears to be protective, whereas disruption of type 5 AC prolongs longevity and protects against cardiac stress. The goal of this review is to summarize the paradigm shift in the treatment of heart failure over the past 50 years from administering sympathomimetic amine agonists to administering β -adrenergic

receptor antagonists, and to explore the basis for a novel therapy of inhibiting type 5 AC.

Keywords Adenylyl cyclase · Longevity · Heart failure · β -Adrenergic receptors

Introduction

Current medical management of heart failure depends upon the degree of heart failure and extent of symptoms as well as the severity of the existing co-morbidities. In general, the current management of heart failure includes the use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers, combined with β -adrenergic receptor (β -AR) blockers, diuretics, aldosterone antagonists, digitalis, nitrates and in cases of severe heart failure implantable cardioverter defibrillators with or without biventricular pacing. Finally, in those patients with severe refractory heart failure, the options are often limited to palliation with or without chronic inotropes, cardiac transplantation, ventricular assist devices or experimental surgeries or drugs. However, this was not the case 50 years ago; at that time the treatment for heart failure consisted of bed rest, diuretics, digitalis, and the use of β -AR agonists. The latter is based on the reasoning that decreased cardiac contractility is a *sine qua non* in heart failure, and it was known that β -AR stimulation is one of the most potent stimulators of cardiac contractility [1–3]. It was also recognized that sympathetic stimulation via the cardiac nerves or via sympathomimetic amines was less effective in heart failure, as established by Braunwald and coworkers [4, 5]. Later Bristow and coworkers identified this desensitization to sympathetic stimulation as due to β -AR down regulation [6, 7]. The initial reasoning underlying therapy with

D. Ho · L. Yan · K. Iwatsubo · D. E. Vatner · S. F. Vatner (✉)
Department of Cell Biology and Molecular Medicine and The
Cardiovascular Research Institute, University of Medicine
& Dentistry of New Jersey, New Jersey Medical School,
185 South Orange Avenue, MSB G609, Newark, NJ 07103, USA
e-mail: vatnersf@umdnj.edu

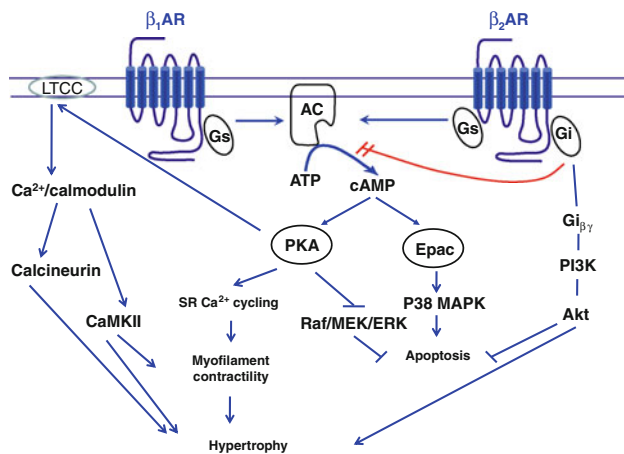


Fig. 1 Schematic representation of β -AR signaling pathway in cardiac myocytes. Upon β -AR stimulation, the $G_s\alpha$ subunit-bound AC produces cAMP which activates target molecules, PKA and Epac. PKA regulates Ca^{2+} homeostasis through SR and LTCC, increases contractility. Under conditions of excessive sympathetic stimulation, PKA-induced Ca^{2+} leads to hypertrophy. On the other hand, activated PKA inhibits Raf, which results in the activation of the MEK/ERK signaling, and thus suppression of apoptosis. In contrast, Epac, the other target protein of cAMP than PKA, induces myocyte apoptosis via the P38MAPK pathway. Upon activation of β_2 -AR, the $G_i\beta\gamma$ subunits are released and activate the PI3K/Akt pathway. Activated Akt inhibits apoptosis, and accelerates hypertrophy

sympathetic agonists was based on the simple concept that these pharmacological agents improved contractility, which was the fundamental derangement in the failing heart.

Physiologically, the activation of the β -AR neurohormonal activity is a compensatory mechanism for the reduction in cardiac output and systemic blood pressure during heart failure. When the β -AR system is activated in the heart, the result is the activation of the associated adenylyl cyclase (AC)—cAMP—protein kinase A (PKA) pathway, which is predominantly mediated through β_1 -ARs. The resulting increase in PKA activity in turn leads to the release of Ca^{2+} from the sarcoplasmic reticulum with consequent increase in inotropy [8–10] (Fig. 1). The activity of cAMP/PKA is kept in check by the activity of cAMP phosphodiesterases (PDE) and protein phosphatases [11–14], and the activation of these enzymes leads to the breakdown of cAMP and inactivation of PKA. As noted earlier, with chronic heart failure one of the key phenotypic changes independent of the etiology of heart failure is the diminished cardiac response to catecholamine stimulation as indicated by the blunted contractile response to β -AR agonists [15–18]. This alteration in catecholamine response appears to be associated with both a decrease in β -AR density and subtype composition [19, 20], an increase in β -AR kinase activity resulting in an increase in the dissociation of β -AR from G_s [21], leading to the impairment of cAMP—PKA signaling pathway [22, 23]. Due to the

decreased cardiac function seen in heart failure and the positive inotropic response seen with β -AR stimulation, it was attractive 30–50 years ago to attempt to overcome this β -AR desensitization and increase cardiac function through the stimulation of the adrenergic system in patients as a way to re-establish hemodynamic stability. However, it was not appreciated until much later that β -adrenergic desensitization in heart failure is actually a compensatory mechanism and accordingly interfering with this mechanism can be deleterious.

Adrenergic receptors

The β -ARs serve as an interface between the adrenergic signaling originating from sympathetic nerves or circulating catecholamines and the cellular response of the end organ. The interest in how the sympatho-adrenal system regulates the heart and blood vessels dates back over 100 years. Epinephrine was first purified in 1899 by Abel, and then in 1906 Dale first introduced the concept of sympathetic receptors based on his observation with the effects of ergot alkaloid antagonists in various organs and blood vessels. He noted ergot alkaloids had stimulatory effects in muscular organs, while having inhibitory effects in structures associated with sympathetic stimulation, and suggested the possible existence of a receptor mechanism for adrenalin [24]. Much work has been done since that time to understand the mechanisms behind this signaling pathway. Prior to 1948 adrenergic signals were thought to be mediated by two fundamentally opposing classes of chemical transmitters, sympathin E and sympathin I, with either excitatory or inhibitory effects [25]. At that time, various types of sympathomimetic amines were used for the study of adrenergic systems including norepinephrine (arterenol), α -methylnorepinephrine (cobefrine), racemic-epinephrine, levo-epinephrine, α -methylepinephrine (methyl-epi), and isoproterenol (*N*-isopropylarterenol). Due to the fact that the then available preparations of adrenalin were fundamentally impure and displayed a wide variability in contents of epinephrine and norepinephrine, the resulting conclusions based on these studies were often difficult to interpret [26]. This was further compounded by the lack of awareness for the existence of subtypes of ARs, resulting in a lack of understanding for the receptor specificity found with the different sympathomimetic amines used.

A major step forward was taken with the work of Ahlquist, who proposed the existence of different types of ARs, which when stimulated by the sympatho-adrenal system would elicit different physiological responses. In 1948, Ahlquist described and characterized these receptors based on the observation that there are two patterns of

response to adrenergic stimulation and he theorized the existence of two types of adrenergic receptors and hence named them α and β -AR [27, 28]. It wasn't until the late 1960s through the early 1990s that the current classification of the existing subtypes of these two major classes of adrenergic receptors, α (α_1 , α_2) and β (β_1 , β_2 , β_3), was fully established. Each of these two major types of receptors can be further subdivided into various subtypes. Currently, six subtypes of α -ARs have been described, α_1 -AR can be divided into α_{1A} , α_{1B} , α_{1D} and the subtypes of α_2 -AR includes α_{2A} , α_{2B} and α_{2C} [29, 30]. Although α -ARs are primarily involved in the regulation of peripheral resistance, there is now substantial evidence that they play a role in regulating cardiac contractility as well [31]. In the heart, α_2 -ARs were found to regulate sympathetic neurotransmission, and the disruption of α_2 -ARs led to increased cardiac hypertrophy and decreased cardiac contractility [32]. The α_{1-} , α_{1A-} and α_{1B-} ARs account for the majority of the cardiac α_1 -ARs [33]. Furthermore, in 2001 Lin et al. demonstrated the ability of α_{1A} -AR overexpression to enhance cardiac contractility in transgenic mice with cardiac-specific overexpression of α_{1A} -AR [34]. There appears to be evidence pointing to the ability of α_{1A} -AR overexpression to protect against myocardial ischemia [35]. Conversely, work in transgenic mice overexpressing α_{1B} -AR showed decreased LV function as well as contractile response to β -AR stimulation, likely due to the activation of Gi [36, 37]. Although α_{1D} -AR accounts for a minor proportion of α_1 -AR in the heart; it does appear to be the predominant α_1 -AR within human coronary arteries [38].

Use of inotropic agents in the treatment of heart failure

The initial enthusiasm for the pharmacologic manipulation of the adrenergic system for the management of heart failure revolved largely around the use of the sympatho-adrenal hormones, epinephrine and norepinephrine, to increase inotropy through stimulation of the β -ARs. However, this initial enthusiasm waned even before the age of controlled clinical trials, since it was readily recognized that patients either became hypertensive or arrhythmic upon catecholamine therapy. This led to the search for synthetic sympathomimetic amines that retained the characteristic of β -AR stimulation to increase cardiac contractility, but are devoid of α -AR effects that lead to increased peripheral resistance, reduced renal flow and hypertension. Isoproterenol was followed by dopamine, the β_1 -AR selective agonist dobutamine and the PDE inhibitor milrinone, all of which turned out to exert adverse outcomes when administered chronically in heart failure [39–45].

Isoproterenol, a sympathomimetic agent structurally similar to epinephrine, functions as a non-selective β_1 - and

β_2 -AR agonist with strong chronotropic and inotropic effects was first used in the treatment of heart failure in the 1960s and was found to elicit fatal arrhythmias [39] as well as increase myocardial ischemia in patients with coronary artery disease [40]. These findings led the pharmaceutical industry to develop β -adrenergic agents that selectively stimulated inotropy without increasing chronotropy or arterial pressure. Interestingly, the adverse actions of increasing chronotropy and arterial pressure were limited in these newer agents by the powerful arterial baroreceptors, which helped maintain pressure and heart rate in the face of sympathomimetic stimulation [46, 47]. Using conscious dogs with arterial baroreceptor denervation in conjunction with muscarinic blockade, Vatner et al. found that sympathomimetic amines stimulated the baroreceptors leading to increased parasympathetic and decreased sympathetic tone, thereby buffering the pressor and chronotropic effects of these sympathomimetic amines. Furthermore, in the absence of these reflexes, the adverse positive chronotropic and pressor effects of the β -AR agonists were unmasked [48]. Therefore, it could be concluded that the absence of the chronotropic and pressor responses, in vivo, was actually due to arterial baroreceptor reflex buffering of these actions.

In the search for a β -AR agonist that did not increase arterial pressure and heart rate, but did improve cardiac contractility, dopamine, a naturally occurring amine with prominent actions in the central nervous system and peripheral vascular beds, was found to have these properties. For example, dopamine, a non-selective β - and α -AR agonist, at low doses (In humans $\leq 2 \mu\text{g/kg/min}$) leads to the activation of dopamine receptors in splanchnic and renal arterial beds and results in vasodilatation of these vessels [49], infusion at intermediate doses (2–5 $\mu\text{g/kg/min}$) results in direct stimulation of the cardiac β -ARs. However, at higher doses both α - and β -ARs are activated by dopamine and this results in the undesirable effects of increases in both heart rate [41] and vascular tone [42]. Although there have been sporadic reports of dopamine's use in heart failure since 1977 [43, 50], clinical trials where dopamine was compared to dobutamine, a β_1 -selective inotropic agent, dopamine led to greater tachycardia, higher aortic blood pressure as well as it was less effective in its ability to lower left ventricular end diastolic pressure when compared to dobutamine [43] and was felt to be inferior to dobutamine for the management of heart failure. Furthermore, dopamine was found to have respiratory depressive effects, it decreased minute ventilation in patients with heart failure [44]. This respiratory depressive effect is an important consideration in hypoxic patients with heart failure. While dopamine has less chronotropic effect compared to isoproterenol, it did induce greater tachycardia when compared to dobutamine; in addition, its

arrhythmogenic potential remained a concern. Furthermore, the decrease in endogenous noradrenaline stores in severe heart failure contributes to the unpredictability of dopamine's effects in heart failure [51].

Although there was some initial success in the early 1980s with the use of the β_1 -selective inotropic agent, prenalterol, in its ability to improve hemodynamics acutely [52, 53], studies involving long-term administration proved it to be detrimental [54]. Furthermore, the observation that prenalterol reduced the maximal and submaximal heart rate responsiveness to exercise led to the suggestion that prenalterol may have β -AR blocking effects [55] and that the observed benefits may be secondary to this β -AR blocking effect. Similar results were seen in other heart failure trials involving other β_1 -AR selective inotropic agents such as xamoterol [56]. The randomized placebo-controlled Xamoterol in Severe Heart Failure Study Group [56] found no significant benefit of oral xamoterol over placebo; however, the study again showed variable agonist and antagonist effects over 24 h on heart rate. The first clear indication that utilization of a β_1 -AR selective inotropic agent in heart failure was detrimental came with dobutamine, a predominately β_1 -AR selective inotropic agent. In heart failure, dobutamine leads to an increase in mortality, and this was demonstrated in the 1999 Flolan International Randomized Survival Trial [57], the results of which were further confirmed by a series of large scale clinical trials [58, 59]. Clinical trials in the 1990s involving the β_2 -AR agonist, dopexamine, showed some initial clinical benefit over placebo [60, 61], however, much of this clinical benefit was attributed to the vasodilatory and cardiac unloading effects of this β_2 -AR agonist.

Since many of the inotropic agents also have vasodilatory effects, it is important to review briefly the use of vasodilators in the treatment of heart failure. It has been known since the 1970s that vasodilators such as nitroprusside can lead to significant left ventricular functional improvement in the setting of heart failure [62–64]. Appropriately vasodilators were compared against inotropic agents, mostly dobutamine, for their clinical efficacy in heart failure. The use of vasodilators was associated with a lower mortality rate when compared to inotropic agents [59]; however, the combined use of inotropes with vasodilators resulted in the highest mortality rate [65]. The use of downstream targets of the β -AR to improve cardiac function has also been explored. Milrinone, a PDE inhibitor that is capable of prolonging the half life of cAMP and thus increasing intracellular Ca^{2+} concentration and enhancing myocardial contractility, was first approved for intravenous use in the late 1980s. In the peripheral vascular system, increases in cAMP result in dilation of both the arterial and venous systems and may lead to subsequent hypotension. Clinical trials involving the use of milrinone

in heart failure found that the use of milrinone led to no clear clinical benefit over placebo in non-ischemic heart failure and can increase mortality in patients with ischemic heart disease [45]. In 1986, the other PDE inhibitor, enoximone, was studied and thought to have a favorable profile for the management of heart failure [66–68]. In 1987, enoximone was compared to dobutamine and nitroprusside in the management of heart failure and found to be superior to both [69]. This clinical improvement was also demonstrated in other small placebo-controlled clinical trials [70–75]. In 1993, the use of enoximone alone and in combination with β -blockade was studied and this combination was found to be synergistic [76], the result was later confirmed in a 1998 study by Shakar et al. [77]. The first signs of increased mortality with the use of enoximone came in 1994, when enoximone was administered to patients with severe end stage heart failure [78]. Then in 2009 results from larger phase III, randomized, double blind placebo-controlled clinical trials involving 1,854 patients at 211 sites in 16 countries (ESSENTIAL-I and ESSENTIAL-II) became available, the trials found no benefit of enoximone treatment over placebo [79], and this finding led to the termination of further development of enoximone.

It became progressively clearer that the use of inotropic agents in the treatment of heart failure resulted in an increase in arrhythmia frequency and myocardial ischemia with associated injury. Furthermore, the potential of direct myocyte toxicity due to intracellular calcium overload is of additional concern. All of these factors mediate the increased mortality observed with the use of inotropic agents in the setting of heart failure.

β -AR receptor overexpression and heart failure

In the mammalian heart, β -ARs predominantly mediate the cardiac response to adrenergic stimulation and thus have been the focus for much of the heart failure research. In the healthy mammalian heart, β_1 -ARs account for the majority (70–80%) of total β -ARs, while β_2 -ARs account for approximately (20–30%) and β_3 -ARs have a minimal contribution [80, 81]. Interestingly, there appears to be a species-specific difference in the physiologic response to β_3 -AR stimulation after autonomic blockade, with a significant response observed in dogs and rodents and a near complete absence of response in primates [82], which is an important consideration for translational research involving animal models.

Paradoxically, there is down regulation of β -ARs in heart failure as a compensatory mechanism elicited to protect the heart from too much sympathetic stimulation. It was noted that in the setting of heart failure the β_1 -AR

undergoes subtype-selective down regulation, resulting in an approximate 50:50 β_1 , β_2 -AR ratio [6, 83], with both sub-types becoming desensitized to adrenergic stimulation secondary to the uncoupling of these ARs from their downstream signaling pathways [84–86]. Again following the reasoning that β -AR signaling is desensitized in heart failure, resulting in the need for higher doses of an agonist to achieve effective increases in myocardial contractility, it was thought that this desensitization of chronic adrenergic stimulation in heart failure could be overcome with genetic overexpression of these receptors. Accordingly, transgenic mice with β_1 -AR, β_2 -AR and Gs α expression were generated, where the receptors would be increased in the heart several fold to allow greater efficacy from a given dose of norepinephrine released from the sympathetic nerves. The initial publications in this field [87–90] were quite promising and the authors suggested that overexpression of β_2 -ARs might be a new treatment for heart failure. It was noted that mice with up to 60-fold overexpression of β_2 -AR showed improvement in cardiac function without cardiac pathology [88, 91]. This was further supported by the gene transfer experiments of Koch et al., where improvement in cardiac response to β -AR stimulation was seen in failing rabbit hearts that received overexpression of the β_2 -AR transgene [89]. It was also noted that in the failing hearts, β -ARs are re-sensitized to adrenergic stimulation after a period of β -AR blockade, and this was characterized by the normalization of β -AR density and subtype distribution [92–94]. Consequentially, the restoration of β_2 -AR was theorized to be a viable therapeutic option for heart failure.

The crucial missing data in the previous animal studies that demonstrated a therapeutic benefit to β -AR overexpression, as well as many others, was that most of the transgenic mice were studied only for a few months, when they were young, and in terms of gene transfer experiments the follow up is generally only for several weeks. Importantly, diametrically opposite conclusions were derived when the transgenic animals were studied for several months to a year. Under those conditions, the transgenic animals developed cardiomyopathy and heart failure. These data in mice parallel the findings in studies in patients, when comparing acute versus chronic effects of sympathomimetic amine therapy. The first evidence of this paradoxical response, i.e., enhanced response to sympathetic stimulation in young adults, but development of cardiomyopathy and heart failure as the animals age, was observed by Iwase et al. in Gs α transgenic mice, showing a shift to the left in response to β -AR stimulation in young mice [95–97], but when the mice were studied several months later it was found that these mice developed cardiomyopathy and heart failure [95, 97–99]. Similar results were found for transgenic mice with overexpression of β_1 -ARs [100] and with overexpression of β_2 -ARs [101].

β_1 -AR overexpression in transgenic mice has been found to induce apoptosis, which was attributed to the associated increase in intracellular calcium and the activation of calcineurin, while β_2 -AR overexpression was initially thought not to lead to the promotion of apoptosis [102–104]. One possible explanation for this difference could be the differences in the downstream effectors of these two major β -ARs. Although it is well accepted that both β_1 - and β_2 -ARs are able to activate Gs α , β_2 -AR have been found to be associated with the inhibitory protein Gi [105–108], capable of inhibiting the main downstream effector, AC, thus raising questions about differences in the regulation of apoptosis by these two β -ARs. Interestingly, when β_2 -ARs are overexpressed in the heart of transgenic mice, apoptosis increases as cardiomyopathy develops [91, 109]. This major difference between in vitro and in vivo studies again points out the importance of verifying in vitro work with in vivo models. It could be that the in vitro work was conducted in young cells, and as noted earlier, and the adverse effects of chronic β -AR stimulation are only observed in older mice. An additional consideration is the duration (12–24 h) of the treatments in the in vitro studies, this short duration maybe insufficient to complete all the changes in protein expression. It is now clear that the overexpression of both β_1 -AR [100] and β_2 -AR [101] leads to development of cardiomyopathy and heart failure, as reflected by decreased cardiac function, increased fibrosis, cardiomyocyte apoptosis and mortality in these transgenic mice (Fig. 2). Further complicating this picture is the differential compartmentation of β_1 -ARs and β_2 -ARs.

Subtype-specific β -AR-induced cAMP compartmentation and heart failure

It was traditionally thought that in the cardiovascular system, both β_1 -AR and β_2 -AR are capable of activating the AC-cAMP-PKA pathway primarily through coupling to Gs proteins. However, there is growing evidence that β_1 -AR and β_2 -ARs elicit different physiological responses upon selective stimulation [110, 111] and mediate distinct signaling pathways [112, 113]. It has been suggested that β_2 -ARs are not only coupled to Gs but also to Gi proteins, which results in different effects from β_1 -AR on activating Ca²⁺ handling, cardiac contractility, and PKA-mediated phosphorylation of proteins that are involved in excitation and contraction coupling, such as phospholamban and myofilament proteins [107, 110, 114]. The inhibitory effect of β_2 -AR stimulation on contractility by coupling to Gi proteins was further confirmed using mice where β_1 - and β_2 -AR were knocked out [113]. Furthermore, previous studies have also suggested that β_1 -AR and β_2 -AR affect apoptosis and myocyte hypertrophy differently, that is,

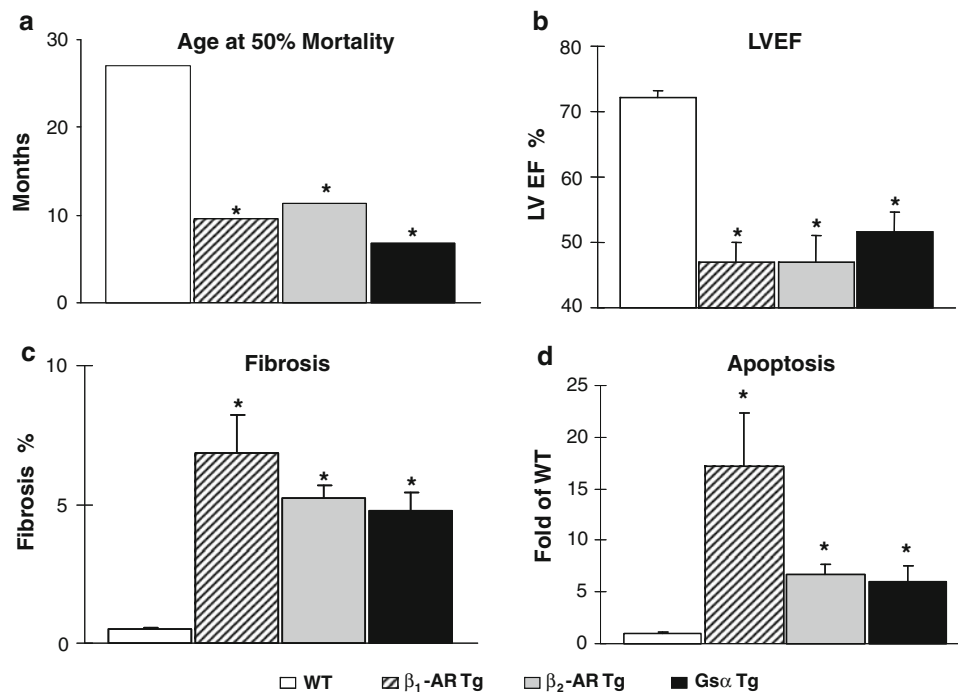


Fig. 2 Effects of cardiac overexpression of β_1 -AR, β_2 -AR or $Gs\alpha$ in mice on development of cardiomyopathy. Panel **a**. The ages at 50% mortality in transgenic mice with overexpression of β_1 -AR (diagonal patterned bar, unpublished results), β_2 -AR (grey bar) [101] or $Gs\alpha$ (black bar) [98] are shown in panel compared against wild type (WT) (C57 [219] or FVB). The time to 50% mortality for the transgenic mice was less than half of that for the respective WT mice

(* $P < 0.05$ compared to WT). Panel **b**. Decreased LV ejection fraction (LVEF) in the Tg animals vs. WT [98, 119]. Panel **c**. Cardiac fibrosis was increased in the Tg animals as compared to WT animals [98, 119]. Panel **d**. Increased cardiac apoptosis in the Tg animals. The percent apoptotic cells in the transgenic mice were normalized against WT [99, 119] (* $P < 0.05$ compared to WT). Figures used and modified with permission

β_1 -AR stimulation induces cardiac myocyte hypertrophy and apoptosis [103, 115] while the activation of β_2 -AR significantly protects the myocardium from apoptosis [116] and hypertrophy [117, 118]. As noted earlier, the concept that β_2 -AR stimulation protects against apoptosis was derived from experiments conducted in young myocytes cultured in vitro. In transgenic mice with cardiac overexpression of β_2 -ARs, Peter et al. found that apoptosis actually increases as they develop cardiomyopathy [119]. This apparent paradox has resulted despite β_1 -AR and β_2 -AR sharing cAMP as a common second messenger, and may be explained by the differentially regulated cardiomyocyte function through the compartmentation of cAMP signaling [120, 121]. However, this spatial compartmentation of cAMP regulated by β_1 -AR and β_2 -AR was not recognized until recently with the emergence of fluorescence resonance energy transfer (FRET) microscopy technique, which was utilized to observe the dynamic changes of cAMP in living cardiomyocytes under β_1 -AR or β_2 -AR stimulation [122, 123]. Furthermore, using combined nanoscale live-cell scanning ion conductance and FRET microscopy techniques, Nikolaev et al. [124] recently demonstrated that the spatial localization of β_1 -ARs and β_2 -ARs are different in normal myocytes, i.e., β_2 -ARs and their induced cAMP signals are localized to the transverse tubules,

whereas β_1 -ARs are widely distributed at the cell crest. However, in the failing heart, the β_2 -ARs were redistributed from the transverse tubules to the cell crest, leading to a change in β_2 -AR associated compartmentation of cAMP. Thus, β_2 -ARs in heart failure behave like β_1 -ARs. Whether the cAMP signals generated by the redistribution of β_2 -ARs are similar to those generated by β_1 -ARs in the regulation of contractility, cell death and apoptosis signaling pathways remain to be determined. In addition, if overexpression of β_2 -ARs induces abnormal compartmentation of cAMP in transgenic mice, this may reconcile the findings of increased apoptosis in chronically enhanced β_2 -AR signaling as with β_2 -AR cardiomyopathy vs. protection in cultured neonatal cells [91, 101, 119]. To prove that abnormal compartmentation of β -ARs is the cause of the observed cardiomyopathy in transgenic mice overexpressing β -ARs, further work is needed for the localization of β_1 -ARs and β_2 -ARs in the mouse heart overexpressing β_1 -ARs or β_2 -ARs.

Downstream effectors of β -adrenergic receptor signaling

Further downstream of the β -AR— $Gs\alpha$ —AC—cAMP—PKA pathway, transgenic animals overexpressing AC (to

be discussed later) and PKA have also been generated. PKA is a cAMP-dependent tetrameric protein consisting of two regulatory and two catalytic subunits [125], where the binding of cAMP to the regulatory subunits results in the disassociation of the regulatory subunits from the catalytic subunits and the activation of the enzyme. Within the mouse model there exist four isoforms of the regulatory subunit (RI α , RI β , RII α , and RII β) and two isoforms of the catalytic subunit (C α and C β), under normal physiologic conditions the major holoenzyme consists of RII β and C α [126]. In 2001, Antos et al. published the results on transgenic mice with PKA C α overexpression, and found the activity of PKA to be elevated, and this was associated with dilated cardiomyopathy, heart failure and increased risk of sudden death, further supporting the adverse effects of chronic β -AR stimulation and PKA activation [127].

As noted earlier, both cardiac overexpressed β_1 -AR and β_2 -AR transgenic mice develop cardiomyopathy [91, 101, 119]. However, the molecular mechanisms mediating the cardiomyopathy in these mice could differ. Recent studies have demonstrated some common as well as different signaling pathways mediated by β_1 -AR and β_2 -AR (Fig. 1). p38 α MAPK was activated in both β_1 -AR and β_2 -AR transgenic hearts, but in contrast to the rescue observed when β_2 -AR transgenic mice were crossed with dominant negative (DN) p38 α mice (bigenic β_2 -AR \times DNp38 α), when β_1 -AR transgenic mice were crossed with DNp38 α mice (bigenic β_1 -AR \times DNp38 α) the cross did not rescue the β_1 -AR overexpression associated cardiomyopathy [119], suggesting other signaling pathways may be more important in ameliorating the adverse effects of chronic β_1 -AR stimulation. For example, mammalian sterile 20-like kinase 1 (Mst1), was found to be up regulated significantly in old β_1 -AR transgenic mice, but not in old β_2 -AR transgenic mice. Our recent studies have demonstrated that type 5 AC (AC5) could be a critical enzyme mediating cardiomyopathy in β -AR transgenic mice and that inhibition of AC5 rescues the cardiomyopathy in these transgenic mice [128].

The results of these studies in transgenic mice with overexpressed β -ARs, Gs α or PKA have further established the pathological effects of chronic adrenergic stimulation and are akin to the studies in patients with heart failure, demonstrating improved cardiac function with short-term inotropic therapy, but exacerbation of heart failure, development of lethal arrhythmias and premature mortality with more chronic therapy [57, 65, 129, 130].

Paradigm shift: advent of β -AR blockade in heart failure

The groundwork for the advent of treating patients with heart failure with β -blockers, rather than agonists, was laid

by the parallel studies noted earlier demonstrating adverse effects of chronic β -AR stimulation in transgenic mice with overexpressed β -ARs or Gs α or PKA along with the studies in patients with prolonged β -AR agonist therapy noted earlier. These studies in combination with the work of Bristow and colleagues demonstrating down regulation of β -ARs in patients with heart failure as a compensatory, protective mechanism, led to the exploration of β -AR blockers in heart failure. β -AR blockade was initially attempted as a therapy to control the tachycardia associated with heart failure, unexpectedly it was found to significantly reduce mortality in patients with heart failure [131, 132]. The potential benefit of β -AR blockade in heart failure was first demonstrated in 1974 by Waggstein in a 7 patient series involving the use of a non-selective β -AR blocker, alprenolol, in the setting of congestive cardiomyopathy [133]. From that point on additional series of case reports and case series continued to emerge, further demonstrating the utility of β -AR blockade in the management of heart failure. However, as debate persisted over the use of β -AR blockade in the management of heart failure, discussions and case reports continued. In 1993, clinical benefits of β -AR blockade were demonstrated in a moderate sized placebo-controlled clinical trial with the β_1 AR selective blocker, metoprolol. The trial involved 383 patients with idiopathic dilated cardiomyopathy and heart failure (MDC trial study) [134]. The MDC trial was followed by another β_1 selective β -AR blocker trial, the Cardiac Insufficiency Bisoprolol Study (CIBIS) in 1994 [135], then came the US Carvedilol (non-selective β/α_1 blocker) Study in 1996 [131, 136]. The first truly large randomized clinical trial was released in 1999, The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II) [137–140] involving 2,647 heart failure patients randomized to either bisoprolol or placebo and followed for a mean duration of 1.3 years, the study was terminated early due to clear evidence of mortality benefit with all cause mortality of 11.8 vs. 17.3% bisoprolol vs. placebo, and a sudden death rate of 3.6 vs. 6.3%. In 2000, the Metoprolol CR/XL Randomized Intervention Trial in congestive heart failure (MERIT-heart failure) study was released. This study involved 3,991 patients randomized to either metoprolol or placebo, and again a significant (19%) reduction in total mortality or all cause hospitalization led to the early termination of the study and demonstrated a clear benefit in using β -AR blockers in the management of heart failure [141]. By this time, the benefit of β -blocker on mortality and use of β -AR blockers in treatment of heart failure had been well established. Current clinical decision in the use of β -AR blockers requires familiarity with the pharmacologic variability among the available β -blockers. In general, β -blockers can be classified as β_1 -AR selective, non-selective β -AR blockers and non-selective β/α -AR

blockers. β_1 -AR selective β -blockers such as atenolol, while able to provide effective blockade of β_1 -AR, leaves vascular α -AR unopposed, and this in turn leads to peripheral vasoconstriction and a reflex increase in aortic pressure as demonstrated by the decreased ability of atenolol to reduce aortic pressure in the CAFÉ study [142]. Non-selective β/α -AR blockers such as carvedilol resulted in vasodilation secondary to α -AR blockade, thus allowing actual lowering of aortic pressure [143, 144]. The use of these non-selective β/α -AR blockers have been tested in clinical trials against placebo in the COPRENICUS trial [145] where 2,289 heart failure patients were randomized to either carvedilol or placebo, and were found to lead to a decrease in mortality (hazard ratio of 0.75), as well as hospitalization (hazard ratio 0.85). When compared to the β_1 -AR selective blocker metoprolol in the COMET trial [146], carvedilol was found to be superior to metoprolol in reduction of all causes mortality (hazard ratio 0.83), without any differences being detected in the incidence of side effects of drug withdrawals. Based on these clinical trials, it is clear that β -AR blockade provides clear mortality benefit in the setting of heart failure, and this mortality benefit may be further enhanced in the setting of vasodilator therapy as in the case of combined β/α -AR blockade.

There were also parallel studies in animal models showing the effectiveness of β -AR blocking agents in heart failure [147]. In addition, the study by Asai et al. [98] demonstrated that propranolol could prevent the cardiomyopathy that develops in Gs α transgenic mice as they age, resulting in normal cardiac function and normal myocardial histology, as well as eliminating the premature mortality (Fig. 3).

It is of interest to note the results of the SENIORS trial [148] where 2,128 patients over the age of 70 with a history of heart failure were randomized to β -AR blocker (nebivolol) or placebo and it was noted that the beneficial effects appear diminished in those patients older than 75.2 years of age. There are several potential explanations for the apparent decreased efficacy in the elderly. First, it may be secondary to higher morbidity and mortality that naturally occur in the older age group and may not be due to decreased benefit or effectiveness of β -AR blockers in this age group. Alternatively, it could be due to β -AR desensitization that occurs in older patients and experimental animals [149–152].

It is also important to note that the beneficial effects of β blockade remains significant despite concurrent use of inhibitors of renin-angiotensin-aldosterone system as indicated in the CARMEN [153] and EPHEsus [154] trials,

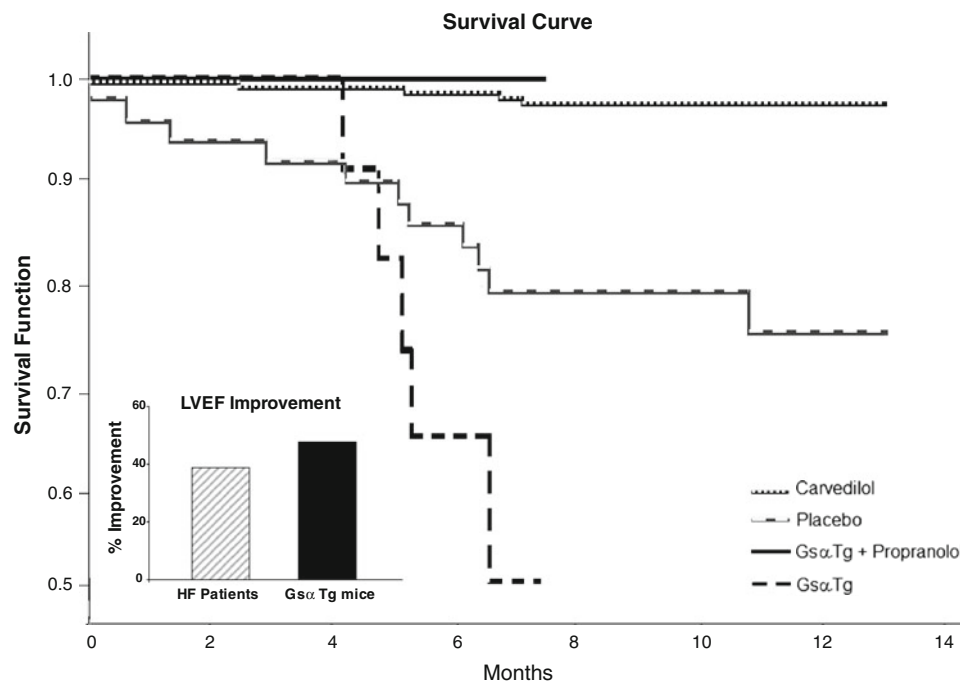


Fig. 3 β -blockade increases survival, and preserves cardiac function in heart failure patients and Gs α Tg mice. Kaplan–Meier survival curve showing mortality improvement in survival in heart failure patients on carvedilol (open dotted line) vs. placebo patients (open broken line) [131] and Gs α Tg mice treated with propranolol (solid black line) vs. vehicle (broken line) [98] indicating prolongation of

lifespan with β -blockade. β -blockade also resulted in improvement in LV function in heart failure patients (diagonal pattern bar) [220] compared against standard therapy and Gs α Tg mice (black bar) [98] compared against placebo as indicated by improved LVEF (left bottom inset) (*, $P < 0.05$ vs. standard therapy vs. placebo). Figures used and modified with permission

thus establishing β blockade as a valuable additional therapeutic option for patients with heart failure despite the concurrent use of other therapeutic agents.

Adenylyl cyclase

Adenylyl cyclase is a transmembrane protein with two hydrophobic and two cytoplasmic domains capable of converting ATP to cAMP upon the stimulation with various G-protein coupled receptors such as β -AR (Fig. 1). Thus far, at least nine mammalian isoforms of AC (AC1-9) have been identified, and each isoform demonstrates a distinct tissue distribution, biological as well as pharmacological properties [155–158]. AC5 and AC6 are the major mammalian AC cardiac isoforms [159, 160]. Furthermore, upon characterization, they were found to be sensitive to and inhibited by low concentration of Ca^{2+} [161, 162]. Interestingly, prior studies suggest that these two isoforms may exert opposite effects on cardioprotection, i.e., overexpression of AC6 appears cardioprotective, whereas, AC5 disruption is also cardioprotective.

Adenylyl cyclase type 5

In 1999, Tepe et al. showed enhanced baseline heart rate and LV function in transgenic mice with cardiac-specific overexpression of AC5 [163]. Furthermore, mating AC5 transgenic with $\text{G}\alpha\text{q}$ transgenic mice was able to rescue $\text{G}\alpha\text{q}$ transgenic mice from LV dysfunction (but not hypertrophy) [164], thus establishing the down regulation of AC5 seen in the transgenic $\text{G}\alpha\text{q}$ overexpressing mice as the dominant pathological mechanism leading to the observed β -AR signaling dysfunction. In direct contrast, Hanoune et al. reported that mice with AC5 overexpression (AC5 transgenic) developed cardiomyopathy with age [155]. Although the original study was not published, the review suggests that mice with AC5 overexpression behave like mice with β -AR overexpression as those seen in $\text{G}\alpha\text{q}$ transgenic [97], β_1 -AR transgenic [100] or β_2 -AR transgenic [101], i.e., hyperfunction at a young age with the development of cardiomyopathy as they age. This finding was confirmed by our observation that AC5 transgenic mice have an increased heart rate and LV ejection fraction at baseline, however under the condition 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 occur [165].

Conversely, mice with AC5 disruption, i.e., knockout (AC5KO), develop resistance to the adverse effects of chronic pressure overload, having the ability to maintain LV function, as well as resistance to the apoptotic process

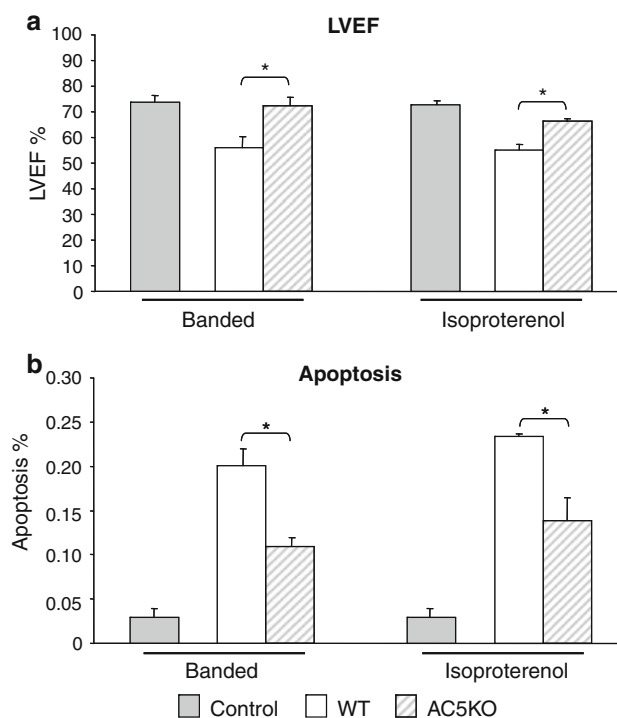


Fig. 4 AC5KO in mice protects against the development of cardiomyopathy. AC5KO (diagonal patterned bar) enhances protection against heart failure induced by pressure overload [166] or catecholamine stress (chronic isoproterenol) [167]. Preserved LVEF (Panel a) and reduced cardiomyocyte apoptosis (Panel b) was observed in AC5KO mice compared with WT in response to catecholamine stress (* $P < 0.05$ compared to no treatment, gray bar) [167]. Figures used and modified with permission

with chronic transverse aortic banding [166]. Furthermore, these AC5KO mice were found to have a more effective physiologic response and desensitization to chronic isoproterenol infusion and a lower number of apoptotic myocytes after chronic stimulation [167] (Fig. 4). This improvement in desensitization to chronic catecholamine stress is a major defense mechanism against the pathogenesis of heart failure [168, 169]. These results speak to the central role of AC5 in the regulation of the apoptosis induction and the subsequent development of heart failure. By the same token, the inhibition of AC5 could prove to be a novel therapy for heart failure.

Adenylyl cyclase type 6

Interestingly, the other major cardiac AC isoform, AC6, most often has been reported to play a protective role in the heart. Hammond et al. demonstrated that mice with cardiac-specific overexpression of AC6 exhibited normal cardiac function at baseline and an increased response to β -AR stimulation, without any signs of physiological or histological abnormality up to 21 months of age [170]. The

same group examined the effects of AC6 overexpression in pigs with intracoronary injection of recombinant adenovirus encoding AC6 or LacZ. They found the treatment with AC6 led to an increase in AC6 protein and stimulated cAMP production, associated with an increase in ventricular function, as demonstrated by increased peak LV dP/dt and cardiac output under isoproterenol challenge in vivo [171]. In 1999 and 2002, Roth et al. demonstrated the ability of cardiac directed AC6 overexpression to rescue mice with cardiomyopathy secondary to Gq overexpression [172, 173]. Then in 2006, the same group found enhanced survival after myocardial infarction in transgenic mice with cardiac-specific AC6 overexpression [174]. Additionally, AC6 deletion led to impaired cardiac cAMP generation and calcium handling that resulted in depressed LV function [175]. Together, these studies indicate that enhancement of AC6 activity could be a potential therapeutic target for the treatment of congestive heart failure. However, a recent study from our laboratory revealed quite different conclusions. We found that AC6 overexpression in mice led to impaired LV function due to chronic aortic banding (4 weeks) compared with wild-type mice, which may have been due to enhanced LV systolic wall stress [176]. It is important to note that previous studies demonstrating the salutary effects of AC6 overexpression did not examine chronic pressure overload [172–174], which was the intervention we studied in our AC6 transgenic mice [176]. Again it could be that distal mechanisms might differ with the stress of chronic pressure overload than that induced by other stresses, e.g., ischemia. However, Tang et al. recently reported chronic aortic banding in female AC6KO mice resulted in improved function [177], which is inconsistent with the previous work from that group on the ischemic interventions [178], but is consistent with our findings of

the adverse effects of chronic pressure overload in AC6 overexpression [176]. However, an appendix in the study by Tang et al. [177] indicated that male AC6KO mice fared worse than wild type with chronic pressure overload.

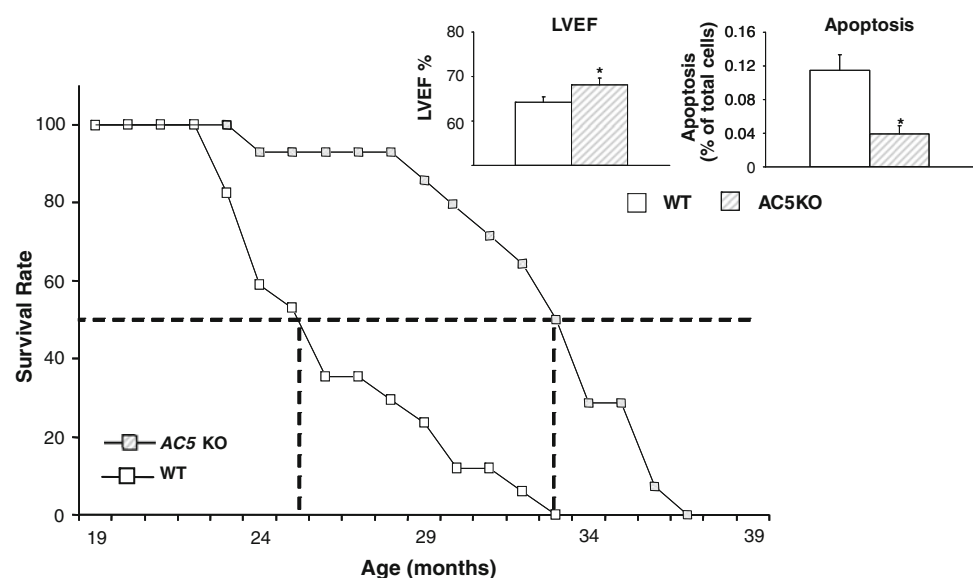
Concept of longevity and stress resistance

It has been recognized for some time that aging and longevity are regulated by evolutionarily conserved molecular pathways [179] and the association between longevity and stress resistance rests with studies utilizing yeast, *C. elegans*, and *Drosophila* as models to study the triggers and the molecular basis for aging and longevity. By utilizing these models, several longevity regulators have been found, including Sir2, an NAD-dependent histone deacetylase, whose increased expression is associated with extended lifespan in yeast [180], *C. elegans* [181, 182] and fruit flies [183–185].

Longevity in mice with AC5 disruption

We have recently reported a novel, genetically engineered animal model, where by AC type 5 isoform is knocked out (AC5KO). AC5KO mice exhibit increased longevity [186] and are protected against cardiac stress related to heart failure [166]. When AC5 is disrupted in this mouse model, phenotypic changes are summarized as [166, 167, 186]: (1) AC5KO mice live a third longer than wild type (Fig. 5). (2) AC5KO mice are protected from aging-induced cardiomyopathy, including decreased left ventricular hypertrophy, a reduction in fibrosis and decreased cardiac apoptosis compared to wild type (Fig. 5). (3) AC5KO mice are

Fig. 5 Disruption of AC5 in mice protects against aging cardiomyopathy. Kaplan–Meier survival curve for AC5KO (diagonal patterned square) shows prolonged lifespan compared with WT (open square), accompanied by preservation of LVEF (left in inset) and a reduction in myocardial apoptosis (right in inset) in the older mice. * $P < 0.05$ vs. WT. [186]. Figures used and modified with permission



protected from aging associated reduction in bone density and fracture risk. (4) AC5KO mice are protected from pressure overload and chronic catecholamine stimulation-induced apoptosis and the associated impairment in cardiac function (Fig. 4). (5) AC5KO mice have increased exercise capacity [187]. (6) Bigenic mice with AC5KO and β_2 -AR overexpression did not develop the typical cardiomyopathy observed in β_2 -AR overexpressing mice, hence rescuing the mice from β_2 -AR overexpression associated cardiomyopathy [128]. (7) Most importantly, AC5KO have several features that resemble caloric restriction, the most widely studied model of longevity. For example, both the AC5KO and caloric restriction models appear to affect metabolism similarly. Both caloric restriction and AC5KO mice weigh less than their control littermates; the caloric restriction mice weighed less because of restricted food intake, however, the AC5KO weighed less despite augmented food intake. In both cases, a decrease in glycogen and blood glucose was observed. (8) Mice on a high fat diet and mice treated with a pharmacologic AC5 inhibitor showed a decreased amount of weight gain and improved glucose tolerance [188]. All of these phenotypic changes in AC5KO mice point to the beneficial effects of AC5 disruption not only in the prolongation of life but also an improvement of health with aging, i.e., improvement in body weight, cardiac function, bone density, glucose tolerance and exercise capacity.

However, the mechanisms mediating the salutary effects of AC5 inhibition in longevity and metabolism are not fully elucidated. Since AC5 is also expressed in the brain, particularly the striatum, it is conceivable that central neural mechanisms may be important either for the beneficial effects of AC5 inhibition or even be responsible for some adverse effects [189–195]. Importantly, the pharmacological AC5 inhibitor we are using (see Future Directions) does not appear to cross the blood–brain barrier [196, 197] and therefore might be useful in unraveling the role of AC5 in the brain.

Although the exact mechanism thorough which AC5 disruption leads to longevity is still unclear at present, resistance to oxidative stress is clearly involved. An upregulation of manganese-superoxide dismutase (MnSOD) [198], a protein with a known role in resistance to oxidative stress, was seen in the AC5KO mice compared to wild-type littermates [186]. The current evidence also suggests that AC5 disruption leads to the activation of the MEK/ERK signaling pathway. The activation of ERK in turn activates MnSOD, which protects against oxidative stress, apoptosis, resulting in cell survival, which leads to the observed longevity in AC5KO mice [186] (Fig. 6). However, this appears to be only part of the story. As noted earlier, one key trait of the AC5KO mice is that they eat more but weigh less than wild-type mice reflecting a favorable difference in metabolic rate compared

to wild-type mice. Thus, similar to caloric restriction models that are dependent on changes in metabolism, it appears that the AC5KO model of longevity may share some of these same mechanisms.

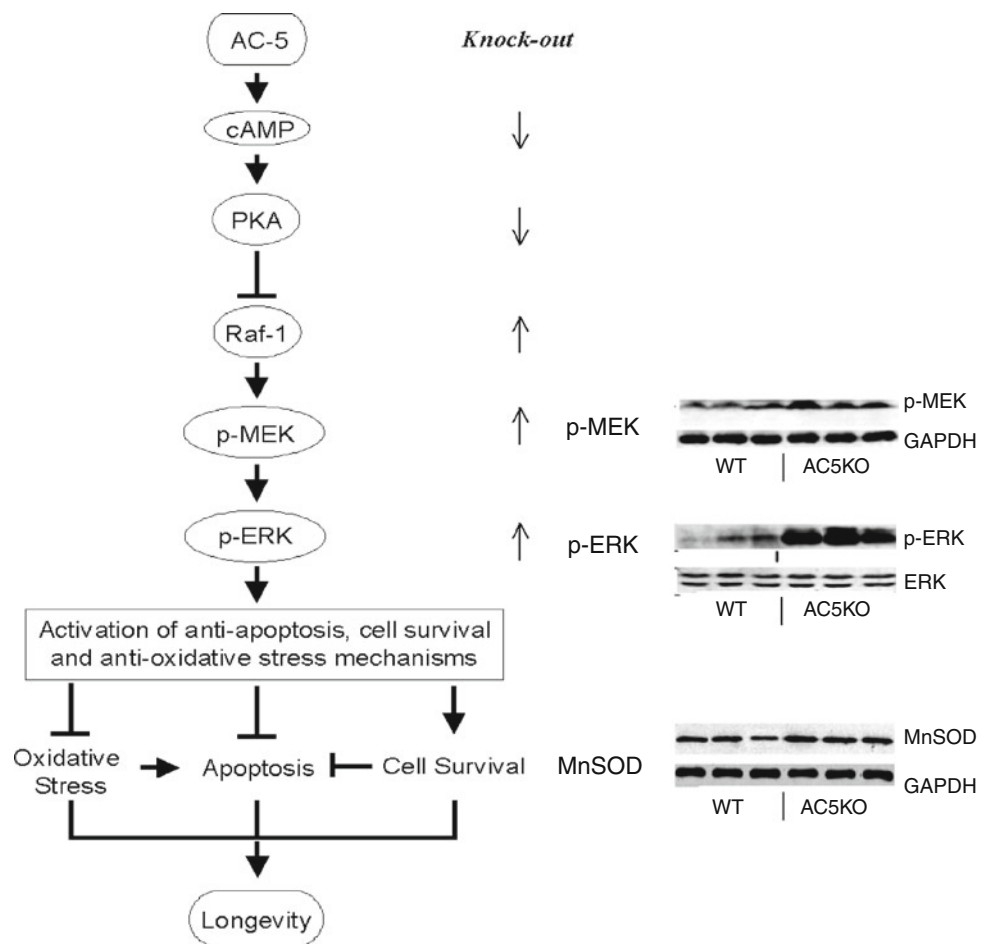
Future directions

The data derived from genetically engineered mouse models suggest that pharmacologic inhibition of AC5 could be a therapeutic strategy for heart failure and longevity. Much attention has been paid to specific AC isoforms that play a major role in the β -AR pathway. The cloning of AC isoforms in 1990s [199–208] enabled studies of specific AC inhibitors. Specific AC5 inhibitors have been developed, based on studies of P-site inhibitors, which are known to inhibit AC [209–211], using purified AC5 in baculovirus [212–218]. However, due to high IC₅₀, low selectivity for AC5 or membrane impermeability of those compounds, it is still premature to translate these compounds to the clinic.

Summary

This review summarized the paradigm shift that occurred over the past half century, where the treatment of heart failure changed diametrically from the use of β -AR agonists to antagonists. The development of a variety of sympathomimetic amines to stimulate cardiac contractility in the failing heart was based on syllogistic reasoning, i.e., that a heart characterized by depressed contractility would benefit from increasing its inotropic state. This concept was supported by numerous experiments demonstrating that catecholamines could restore LV function acutely in hearts with cardiac depression and by studies in transgenic mice with cardiac overexpression of β -AR, where cardiac contractility was enhanced. An important take home message from this review is that the pathophysiology of any disease state in animals or patients, e.g., heart failure, is not simple but rather is exceedingly complex. We know now that distal signaling pathways and compartmentation of β -ARs and AC may modify dramatically the effects of β -AR agonists in heart failure. Based on studies in patients with heart failure on chronic sympathomimetic amine therapy demonstrating increased mortality and morbidity, and based on studies in transgenic mice overexpressing components of the β -AR signaling pathway, which developed cardiomyopathy as they age, a paradigm shift emerged resulting in a diametrically different direction of therapy, i.e., β -AR blockade. However, even though β -AR blockers have improved therapy in patients with heart failure, there

Fig. 6 Mechanism of AC5-mediated regulation of longevity. Disruption of AC5 activates the MEK/ERK signaling pathway [186]. The activation of ERK leads to increased expression of manganese superoxide dismutase (MnSOD), which results in inhibition of apoptosis. On the other hand, the ERK signaling activates the cell survival signaling. Taken together, these mechanisms contribute to increased longevity in AC5KO mice. Western blots demonstrate the changes of key enzymes in the heart by disruption of AC5 [186]. Figures used and modified with permission



is still significant room for further improvement. Our hypothesis is that a new direction will emerge involving distal components of the β -AR signaling pathway. Our recent work suggests that inhibition of type 5 AC is one such mechanism that could develop into a novel therapeutic approach for heart failure.

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