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Neurohumoral activation in heart failure: the role of adrenergic receptors

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ABSTRACT

Heart failure (HF) is a common endpoint for many forms of cardiovascular disease and a significant cause of morbidity and mortality. The development of end-stage HF often involves an initial insult to the myocardium that reduces cardiac output and leads to a compensatory increase in sympathetic nervous system activity. Acutely, the sympathetic hyperactivity through the activation of beta-adrenergic receptors increases heart rate and cardiac contractility, which compensate for decreased cardiac output. However, chronic exposure of the heart to elevated levels of catecholamines released from sympathetic nerve terminals and the adrenal gland may lead to further pathologic changes in the heart, resulting in continued elevation of sympathetic tone and a progressive deterioration in cardiac function. On a molecular level, altered beta-adrenergic receptor signaling plays a pivotal role in the genesis and progression of HF. beta-adrenergic receptor number and function are decreased, and downstream mechanisms are altered. In this review we will present an overview of the normal beta-adrenergic receptor pathway in the heart and the consequences of sustained adrenergic activation in HF. The myopathic potential of individual components of the adrenergic signaling will be discussed through the results of research performed in genetic modified animals. Finally, we will discuss the potential clinical impact of beta-adrenergic receptor gene polymorphisms for better understanding the progression of HF.

Key words: heart failure, sympathetic nervous system, adrenergic receptors.

INTRODUCTION

Heart failure, a syndrome of poor prognosis, is developed as a consequence of cardiac disease, and recognized clinically by a constellation of signs and symptoms produced by complex circulatory and neurohormonal responses (Packer 1992, Katz 2002, 2003). Concisely, heart failure can be described as an inability of the heart to maintain adequate blood circulation in peripheral tissues and the lungs. It is a common endpoint for many forms of cardiovascular diseases, and a major clinical and public health problem. More than 20 million people worldwide are estimated to have heart failure (Cleland et al. 2001, Tendera 2004), and its increasing prevalence is associated with the rise in the median life span of the population (Bonneux et al. 1994). In Brazil, heart failure leads to approximately 25,000 deaths per year (Albanesi Filho 2003), and is associated with considerable morbidity, since patients

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with heart failure undergo frequent hospital readmissions, which are higher in those of lower socioeconomic strata (Albanesi Filho 2003).

Throughout most of the 20th century, heart failure has been regarded as a hemodynamic disorder. According to this view, the impaired pump performance led to increased pulmonary and venous pressures, decreased cardiac output associated with progression of underlying disease, and ultimately to the death of the patient (Katz 2001). By attempting to improve the hemodynamic derangements, the goals of heart failure therapy were to lower the increased venous pressure with diuretics, to unload the failing heart by using peripheral vasodilators and to increase cardiac contractility by administering inotropic agents.

Unfortunately, clinical trials performed with diuretics, peripheral vasodilators and inotropic agents obtained disappointing results, since in spite of causing a short-term hemodynamic improvement, long-term use failed to prolong survival in heart failure patients (Packer 1992, Katz 2002, 2003).

The importance of non-hemodynamic mechanisms in inducing cardiac failure emerged in the early 1980s, when the neurohumoral response to low cardiac output was found to have a major adverse effect on long-term survival (Francis et al. 1984). This finding highlighted the importance of prognosis in heart failure, which had been overlooked until then. More interestingly, the understanding of heart failure passed through a paradigm shift, and currently the syndrome is viewed primarily as a neurohumoral disorder.

The sympathetic nervous system is a critical component of neurohumoral response observed in heart failure. In the early stages of the syndrome, an intrinsic decrease in myocardial function leads to an increase in sympathetic activity. Acutely, through the activation of cardiac beta-adrenergic receptors (β-adrenergic receptors), heart rate and cardiac contractility are increased and compensate for decreased cardiac output, which is returned to a more idealized level. However, as heart failure worsens, sympathetic activity is further increased in an attempt to compensate for a progressive loss of cardiac function. Unfortunately, chronic exposure of the heart to elevated levels of catecholamines released from sympathetic nerve terminals and the adrenal gland may lead to further pathologic changes in the heart, resulting in continued elevation of sympathetic tone and a progressive deterioration in cardiac structure and function (Post et al. 1999, Port and Bristow 2001, Brum et al. 2002, Lohse et al. 2003a).

It has long been suspected that increased activity of the sympathetic nerves is present in heart failure (Starling 1897, Chidsey and Braunwald 1966). In fact, sympathetic nerve activity bears a direct relationship to both severity and prognosis of the heart failure (Cohn et al. 1984, Negrão et al. 2001) (Fig. 1). Likewise, the cardiotoxic effects of catecholamines have been recognized since the beginning of the 20th century (Josue 1907). However, the molecular mechanisms underlying these cardiotoxic effects are just beginning to be understood. Cardiac deleterious effect of sympathetic overactivity seems to be related mainly to the activation of β_1AR pathway (Communal et al. 1999, Lohse et al. 2003a, Xiao et al. 2004). Accordingly, there has been considerable interest in the possibility that therapy directed at βAR and adrenergic signaling pathway has the potential to treat the pathophisiologic mechanisms involved in the progression of the heart failure.

In the present review, the long-term consequences of sustained adrenergic activation in the context of heart failure will be reviewed. We will present an overview of the normal βAR pathway in the heart and then discuss the alterations in β-adrenergic receptor signaling in heart failure. Data from genetic modified animals that demonstrate the myopathic potential of individual components of the adrenergic signaling will also be emphasized. Finally, we will discuss the potential clinical impact of β-adrenergic receptor polymorphisms for better understanding the progression of cardiovascular diseases, such as heart failure.

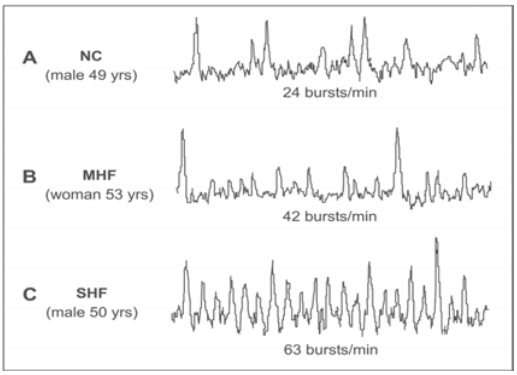


Fig. 1 – Muscle sympathetic nerve activity (MSNA) from control (NC, A), mild (MHF, B) and severe (SHF, C) heart failure patients at rest. MSNA was directly measured from the peroneal nerve using the microneurography technique. Note that resting MSNA was greatest in the patients with severe heart failure (Data from Cardiovascular Rehabilitation and Exercise Physiology Laboratory, Heart Institute, Medical School, Universidade de São Paulo).

THE CARDIAC β-ADRENERGIC PATHWAY

Adrenergic receptors form the interface between the sympathetic nervous system and cardiovascular system. As mentioned above, cardiovascular function is tightly regulated by the sympathetic nervous system. In response to a variety of stimuli, including exercise and blood loss, an increase in cardiac output is met by a commensurate increment in sympathetic nervous system that through β-adrenergic receptors increase the heart rate (chronotropism), the force cardiac contraction (inotropism), the rate of cardiac relaxation (lusitropism), and automaticity.

Ahlquist first classified the adrenergic receptors in 1948 as α (for excitatory) and β (for inhibitory) based on their control of blood vessel contractility (Ahlquist 1948). His initial observations suggested that α-adrenergic activation, for example, generally lead to smooth-muscle contraction, as evidenced by vasoconstriction or uterine contraction, whereas β-adrenergic stimulation produced the opposite effect of relaxing smooth muscle. Ahlquist's classification was expanded by Lands and collaborators (1967), who recognized that both α- and β-adrenergic receptors could be further categorized into 2 distinct subtypes based on their relative potencies for the ligands available at that time. More recently, molecular cloning has led to the identification of 9 adrenergic receptor subtypes, namely α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, and β_3 (Bylund et al. 1994).

In the heart, β_1- and β_2-adrenergic receptor subtypes are expressed at a ratio of 70:30, and both increase cardiac frequency and contractility (Wallukat 2002) (Fig. 2). In addition, β_3-adrenergic receptors have been described to mediate negative inotropic effects in cardiac myocytes (Devic et al. 2001).

All three β-adrenergic receptors subtypes are members of the large family of seven membrane-spanning, GTP-binding protein (G-protein)-coupled receptors. Their activation by an agonist catalyze the exchange of GTP for GDP on the Gα-subunit of G proteins, resulting in the dissociation of the heterotrimer into active Gα- and G$\beta\gamma$-subunits, which are competent to signal independently (Lefkowitz et al. 2002, Wallukat 2002). The heterogeneity of G-protein α subunits of which there are $\sim$20 subtypes (Gs, Gi, Gq, Go, etc) is a central basis of G-protein coupled receptor signaling (Morris and Malbon 1999). Additional levels of signaling specificity are conferred by combinatorial permutations of various $\beta\gamma$-heterodimeric subunits (5β and 11γ subunits) with the α subunits. Even though all three subtypes of β-adrenergic receptors are expressed in cardiac myocyte, they possess distinct intracellular signaling pathways and functional properties (Lohse et al. 2003b, Xiang and Kobilka 2003b). The positive chronotropic effects of β_1 receptor activation are clearly mediated via the stimulatory G protein (G_s) in myocytes. Even though it has been recently proposed that $\beta_1$adrenergic receptor can switch from G_s to G_i-coupling in a PKA-dependent manner (Martin et al. 2004), the intracellular effect of this switching is not known. In contrast, dual coupling of β_2 receptors to G_s and inhibitory G protein (G_i) is evident in cardiac myocytes from new-

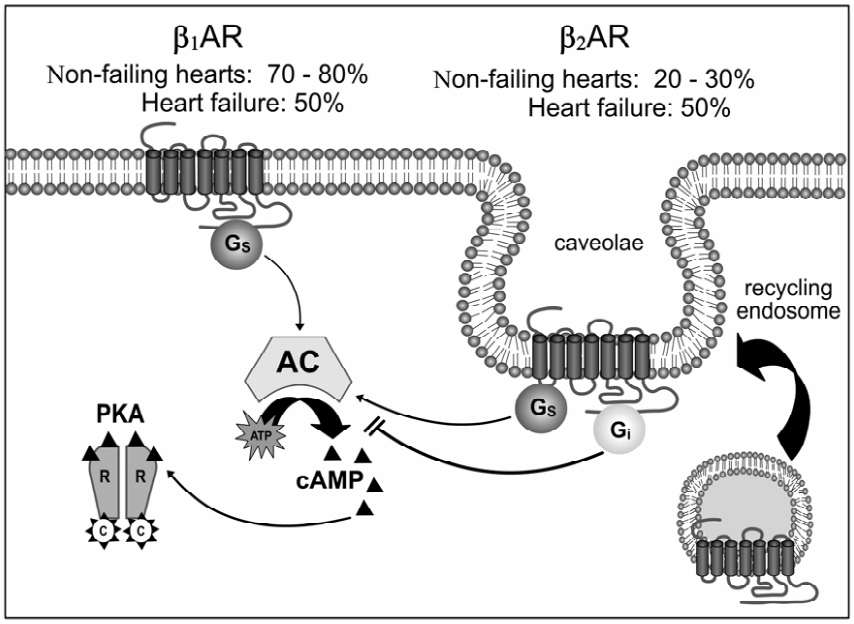


Fig. 2 – Distinct intracellular signaling pathways and subcellular localization of β_1 and β_2 adrenergic receptors (β_1AR and β_2AR) in cardiomyocytes. β_1ARs mediate chronotropic and inotropic effects of catecholamines via the stimulatory G protein (G_s). β_2ARs are normally confined to caveola in cardiomyocyte membranes and this localization is essential for its physiological signaling. β_2ARs mediate transient increase in contraction rate of cardiomyocytes via G_s. However, β_2ARs also couple to inhibitory G protein (G_i), which results in antiapoptotic effects on cardiomyocytes. AC, adenylyl cyclase; cAMP, cyclic AMP; PKA, cAMP-dependent protein kinase A.

born mice (Kuschel et al. 1999, Xiao et al. 2003). The β_2 receptor coupling to G_i is reported to be involved in its anti-apoptotic properties in cardiac myocytes (Communal et al. 1999, Zhu et al. 2001). In neonatal cardiac myocytes from β_1 and β_2 receptor double knockout mice, dual coupling of the β_3 subtype to both G_s and G_i, with the G_i component dominating, has been described (Devic et al. 2001). These specific signaling properties of β receptor subtypes have been linked to subtype-selective association with intracellular scaffolding and signaling proteins, and distinct subcellular localization (Steinberg 1999, Hall and Lefkowitz 2002, Xiang et al. 2002, Xiang and Kobilka 2003a).

Figure 3A is a schematic representation of β-adrenergic receptor signaling in the heart. β-adrenergic receptors stimulate the effector enzyme, adenylyl cyclase (AC) of which there are at least 9 isoforms, being AC's V and VI the main isoforms expressed in the heart. Stimulation of AC results in catalysis of ATP into the second messenger adenosine 3', 5'-cyclic monophosphate (cAMP), which in turn binds to the regulatory subunits of cAMP-dependent protein kinase (PKA). In doing so, the catalytic subunits of PKA are rendered competent to phosphorylate several intracellular protein targets at serine and threonine residues. In several tissues, including the heart, PKA and its targets are in close proximity because of A-kinase anchoring proteins (AKAPs). An association of β_2-adrenergic receptors and AKAPs have been previously reported (for review see Wong and Scott 2004), however

there is a lack of information about the interaction of β_1-adrenergic receptors and AKAPs in cardiac myocytes. Clearly, unique interaction between β_1 and β_2 adrenergic receptors and specific AKAPs would give an additional support to specific signaling properties of these subtypes.

Besides playing a very important role phosphorylating β-adrenergic receptors, which results in partial uncoupling and desensitization of the receptor to further agonist stimulation (heterologous desensitization) PKA has other roles in adrenergic receptor signaling. Some prominent targets of PKA phosphorylation in the adrenergic receptor signaling pathway are: a) L-type calcium channels and ryanodine receptors, both leading to an increase in Ca^{2+} entry into the cells (Zhao et al. 1994, Gerhardstein et al. 1999); b) phospholamban, a modulator of the sarcoplasmic reticulum associated ATP-dependent calcium pump (SERCA), which accelerates Ca^{2+} reuptake by the sarcoplasmic reticulum resulting in an accelerated cardiac relaxation (Simmerman and Jones 1998); and c) troponin I and myosin binding protein-C (MyBP-C), which reduce myofilament sensitivity to Ca^{2+} (Sulakhe and Vo 1995, Kunst et al. 2000, Xiao 2000). Lastly, PKA phosphorylates β-adrenergic receptors, resulting in partial uncoupling and desensitization of the receptor to further agonist stimulation (heterologous desensitization).

As described above the stimulation β-adrenergic receptors leads to dissociation G-proteins in Gα and G $\beta\gamma$ subunits. One important function of the dissociated G $\beta\gamma$ subunit is to facilitate the juxtaposition of β-adrenergic receptors and G-protein receptor kinases (GRK2 also known as β-adrenergic receptor kinase 1, βARK1), which ultimately mediates phosphorylation of β-adrenergic receptors and further desensitization in an agonist occupancy-dependent manner (homologous desensitization).

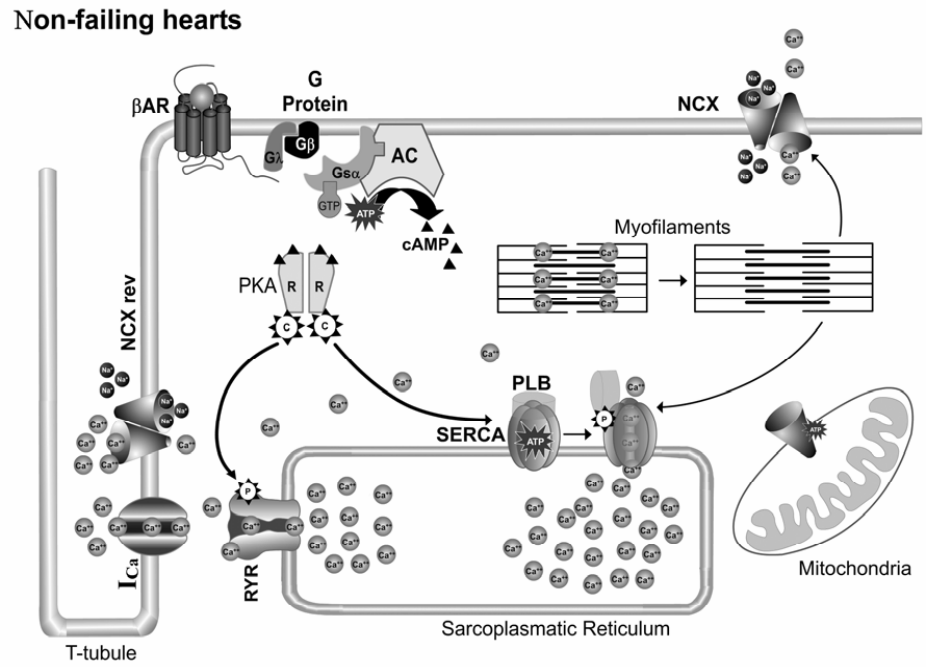
Finally, β-adrenergic receptors in cardiac myocytes can regulate other effectors independently of AC activation, including voltage-sensitive calcium channels and sodium channels (Reiter 1988, Kaumann 1991, Matsuda et al. 1992).

THE CARDIAC β-ADRENERGIC PATHWAY IN HEART FAILURE

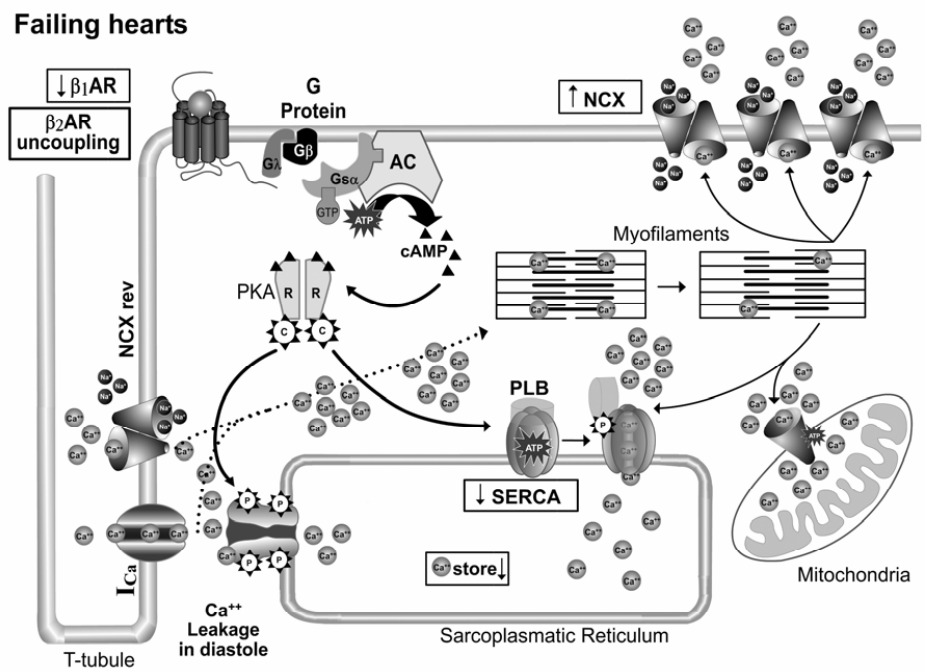
Heart failure caused by diverse etiologies is characterized by a sympathetic hyperactivity, paralleled by a reduction in β-adrenergic receptor density, and desensitization of remaining β-adrenergic receptor, leading to a markedly blunted cardiac contractile response to β-adrenergic receptor activation (Bristow et al. 1982) (Fig. 3B). A reduction in β-adrenergic receptor density was first demonstrated in 1982 by Bristow et al. (Bristow et al. 1982) in failing hearts explanted at the time of transplantation. In addition, they also showed β-adrenergic receptor desensitization in the setting of heart failure. Alterations in β-adrenergic receptor signaling have been also observed at Gi, AC and GRK2 (Post et al. 1999) levels.

As previously described, β_1-adrenergic receptor subtype comprises approximately 70 to 80% of total cardiac β-adrenergic receptors in non-failing hearts. In heart failure, β_1-adrenergic receptor is selectively down-regulated resulting in an approximate 50:50 ratio of β_1 to β_2 subtypes (Wallukat et al. 2003). In addition, β_2-adrenergic receptor seems to be uncoupled from activation of AC (Post et al. 1999, Port and Bristow 2001, Lohse et al. 2003b). This latter effect seems to be due to β-adrenergic receptor phosphorylation by specific kinases, namely: a) GRK2 (βARK1) that phosphorylates both β-adrenergic receptor subtypes in an agonist-dependent manner, and b) PKA and PKC that phosphorylate β-adrenergic receptors in an agonist-independent manner (Sibley et al. 1986, Hausdorff et al. 1990). Interestingly, elevated levels of GRK2 in failing human hearts have also been reported (Ungerer et al. 1993).

Sustained β-adrenergic receptor activation can also influence G protein and AC expression. It has been demonstrated that Gi expression levels are increased in human failing hearts of different etiologies (Feldman et al. 1988, Neumann et al. 1988, Eschenhagen et al. 1992, Ping and Hammond 1994) leading to a decreased Gs:Gi ratio. Likewise, AC's


Non-failing hearts
βAR
G Protein
Gβ
Gα
Gsα
GTP
AC
ATP
cAMP
NCX
Myofilaments
PKA
R
R
C
C
NCX rev
PLB
SERCA
P
ATP
Mitochondria
I_{Ca}
RYR
T-tubule
Sarcoplasmatic Reticulum

A)


Failing hearts
↓β1AR
β2AR uncoupling
G Protein
Gβ
Gα
Gsα
GTP
AC
ATP
cAMP
↑ NCX
Myofilaments
PKA
R
R
C
C
NCX rev
PLB
P
ATP
↓ SERCA
store↓
Mitochondria
I_{Ca}
Ca++ Leakage in diastole
T-tubule
Sarcoplasmatic Reticulum

B)

←

Fig. 3 – Excitation-contraction (EC) coupling in non-failing (A) and failing (B) hearts. (A) In non-failing hearts during systole EC coupling involves depolarization of the transverse tubule (T-tubule), which activates voltage-gated L-type Ca^{++} channels (I_{Ca}) in the plasma membrane. Additional Ca^{++} influx can occur through reverse-mode Na^{+}/Ca^{++} exchanger (NCX rev). Ca^{++} influx via I_{Ca} triggers Ca^{++} release from the sarcoplasmatic reticulum (SR) via ryanodyne channels (RYR). During diastole, intracellular Ca^{++} is pumped out of the cytoplasm by the SR Ca^{++}ATPase (SERCA), which is regulated by Phospholamban (PLB). The 'P' on PLB indicates that when phosphorylated, PLB release SR inhibition. In addition, Ca^{++} is extruded from the cell by the sarcolemmal NCX. The β-adrenergic receptor (βAR) activation increases EC-coupling gain during systole and diastole through phosphorylation, via protein kinase A, of I_{Ca}, RYR, PLB. (B) In failing hearts EC-coupling altered. RYR are hyperphosphorylated by PKA, which leads to greater sensitivity to Ca^{++} induced Ca^{++} release at low and moderate cytoplasmic Ca^{++} concentrations. The long-term effect of PKA hyperphosphorylation of RYR is an increased open probability at low intracellular Ca^{++} concentrations, consistent with Ca^{++} leakage during diastole. In addition SERCA is downregulated, while NCX is upregulated in failing hearts, which contributes to depletion of SR Ca^{++} stores.

V and VI isoforms were reported to be downregulated both in mRNA and protein levels of failing hearts (Ishikawa et al. 1994). Collectively, β-adrenergic receptor downregulation and desensitization, as well as decreased Gs:Gi ratio and AC's V and VI isoform expression will culminate with less production of cAMP. Decreased formation of cAMP, in turn, leads to diminished activation of PKA. However, decreased PKA activity is not always correlated with less phosphorylation of its intracellular effectors in failing hearts. For example, the cardiac ryanodine receptor (RyR_2), a calcium release channel localized in sarcoplasmic reticulum and target of calcium-induced calcium release triggered by L-type calcium channels, has been shown to be hyperphosphorylated in failing hearts (Marx et al. 2000, Reiken et al. 2003, Wehrens et al. 2005). The hyperphosphorylated RyR_2 is associated with a dissociation of FKBP12.6 (a protein which stabilizes the closed state of RyR_2 channels) from RyR_2 channels, and results into calcium leakage during diastole, and further depletion of sarcoplasmic reticulum calcium stores (Fig. 3B). The hyperphosphorylated state of RyR_2 receptors is likely due to the downregulation of phosphatases PP1 and PP2A association with RyR_2 (Reiken et al. 2003, Wehrens and Marks 2003). In a similar manner, PKA phosphorylation of phospholamban appears to be unchanged in heart failure (Bohm et al. 1994, Kirchhefer et al. 1999), whereas its overall phosphorylation seems to be decreased (Fig. 3B) (Schwinger et al. 1999). The decreased phosphorylation of phospholamban results in a greater inhibition of the sarcoplasmic reticulum calcium ATPase (SERCA2), and thereby in a decreased cardiac relaxation. Concomitantly to decreased phospholamban phosphorylation, SERCA2 expression both at mRNA and protein levels seems to be decreased in heart failure leading to an additional impairment of Ca^{2+} reuptake to sarcoplasmic reticulum and consequently diastolic dysfunction (Fig. 3B) (Hajjar et al. 1998, Schwinger et al. 1999). Together all changes in β-adrenergic receptor signaling pathways described above result in decreased cardiac inotropic and lusitropic responses to adrenergic stimulation.

Abnormalities in β-adrenergic receptor signal transduction are not involved only in cardiac functional impairment, they also play a role in cardiac structural changes observed in heart failure being involved in the transition from compensated cardiac hypertrophy to decompensated heart failure (Morisco et al. 2001, Lowes et al. 2002). The exposure to high levels of circulating cathecholamines has been reported to be toxic to cardiac myocytes (Rona 1985, Mann et al. 1992), leading to myofibrillar degradation and increased cardiac collagen volume fraction mediated by β-adrenergic receptor stimulation. However, substantial evidence from

the literature points to significant differences between β_1 and β_2-adrenergic receptor subtypes and their ability to stimulate apoptosis, or programmed cell death, in isolated cardiac myocytes (Xiao et al. 2004) and *in vivo* experiments performed in knockout mice lacking β_1, β_2 or both subtypes (Patterson et al. 2004). β_1-adrenergic receptor stimulation results in an increased cardiac myocyte apoptosis via cAMP-dependent mechanism (Communal et al. 1998, 1999), whereas stimulation of β_2-subtype inhibits apoptosis via a Gi-coupled pathway involving PI3K and Akt-PKD (Chesley et al. 2000, Zaugg et al. 2000, Zhu et al. 2001). These findings have interesting clinical implications for heart failure therapy, since they provide cellular and molecular mechanisms that underline the beneficial therapeutic effects of some β-adrenergicreceptor antagonists, and provide the rationale for combining β_1-subtype specific blockade with β_2- subtype activation.

MYOPATHIC POTENTIAL OF INDIVIDUAL COMPONENTS OF ADRENERGIC RECEPTOR PATHWAYS

The advances in mice engineering technologies and a better knowledge of the genome structure have provided a wealth of information with regard to understanding the role of adrenergic receptors signaling in heart failure. Moreover, mice have proven to be a valid model for studying heart failure because of the similarities between this disorder in mice and humans (Chien 1996). In this section we briefly describe some data obtained from both the "gain of function" (transgenic animals) and "loss of function" (knockout animals) of individual cardiac genes in an attempt to better understand the molecular mechanisms underlying the adrenergic receptor pathways in heart failure. Table I summarizes the genetically altered mouse models that we will discuss.

Adrenergic Receptors

The notion that β_1- and β_2-adrenergic receptor signaling and functional properties are distinctly different has been emphasized by studies performed in transgenic mouse models. Mice overexpressing the β_2-subtype at relatively high abundance ($\sim$50 to 200 fold increases in receptor numbers) produced no or limited histopathology at time points up to four months of age while maintaining a hyperdynamic state characterized by increased basal AC activity, enhanced cardiac contractility and left ventricle function (Milano et al. 1994). The gene dose effect of the overexpression of the β_2-subtype in a study by Liggett (Liggett 2000a) has provided data that showed a expression level dependence with regard to the development of cardiomyopathy. Animals expressing more than 60 times the β_2-subtype maintained their hyperdynamic state for more than one year, without an apparent increase in mortality. In contrast, overexpression levels above 100 fold resulted in progressive cardiac enlargement, the development of heart failure, and premature death.

In marked contrast to results obtained from β_2-subtype that needs a high level of expression to develop of heart failure, mice overexpressing β_1-subtype, as low as five fold the endogenous expression level, present progressive hypertrophy and ventricular dysfunction, which culminate with heart failure by the age of 35 weeks (Engelhardt et al. 1999). The pathways mediating the cardiac deleterious effects of β_1-subtype overexpression seems to involve an altered calcium handling (Engelhardt et al. 2001, 2004) and increased Na^+-H^+ exchanger (Engelhardt et al. 2002). In addition, overexpression of β_1-subtype in mice leads to upregulation of pro-apoptotic proteins, such as Bax (Bisognano et al. 2000), and chronic stimulation of β_1-subtype has been shown to increase rate of apoptosis (Communal et al. 1998, Xiao 2001, Zhu et al. 2001). Taken together these results recapitulate findings obtained from selective stimulation of β_1-and β_2-subtypes in cardiac myocytes culture, where β_1-subtype seems to induce apoptosis whereas β_2-subtype is antiapoptotic.

The role of myocardial adrenergic signaling in cardiac function has been additionally explored in transgenic hearts overexpressing β-adrenergic re-

TABLE I

The genetically altered mouse models in an attempt to better understand the molecular mechanisms underlying the adrenergic receptor pathways in heart failure.

Gene	Genotype	Cardiac Phenotype	References
$\beta 1-$AR	Overexpression	Cardiac hypertrophy and progressive heart failure	Engelhardt et al. 1999
	$\beta 1-$AR (-/-)	Majority die prenatally	Rohrer et al. 1996
$\beta 2-$AR	Overexpression	Enhanced contractile function or progressive dilated cardiomyopathy (at high level)	Milano et al. 1994, Liggett 2000a
	Thr 164IIe mutant	Decreased heart rate and cardiac function and blunted responses to isoproterenol	Turki et al. 1996
$\beta-$ARK1	Overexpression	Reduced functional coupling of β-AR	Koch et al. 1995
	$\beta-$ARK1 (-/-)	Embryonic lethality because of ventricular hypoplasia and heart failure	Jaber et al. 1996
$\beta-$ARKct	Overexpression	Enhanced cardiac contractility	Koch et al. 1995
α_{1B}-AR	Overexpression	Cardiac hypertrophy and dilated cardiomyopathy	Akhter et al. 1997, Zuscik et al. 2001
$\alpha_{1A/1B}$-AR	α_{1A}-AR (-/-) and α_{1B}-AR (-/-)	Reduced cardiac growth after birth and functional alterations observed in heart failure	McCloskey et al. 2003, O'Connell et al. 2003, Turnbull et al. 2003
$\alpha_{2A/2C}$-AR	α_{2A}-AR (-/-) and α_{2C}-AR (-/-)	Elevated sympathetic tone and decreased cardiac function	Hein et al. 1999, Brum et al. 2002
G_s protein	Overexpression	Tachycardia basal, altered β-AR density, increased frequency of cardiac arrythmias, and myocyte hypertrophy apoptosis and fibrosis	Iwase et al. 1996 Lader et al. 1998, Geng et al. 1999
G_i protein	Overexpression	Contributor to β-AR dampened signaling in cardiac hypertrophy and failure	Rau et al. 2003, Janssen et al. 2002
	Modified G_i coupled receptor (Ro1)	Developed lethal cardiomyopathy	Redfern et al. 2000
G_q protein	Overexpression	Exhibit a myopathic phenotype with cardiac hypertrophy and fibrosis	D'Angelo et al. 1997, Sakata et al. 1998
ACV	Overexpression	It does not induce any form of cardiomyopathy	Tepe and Liggett 1999
ACVI	Overexpression	It does not induce any form of cardiomyopathy	Roth et al. 1999

ceptor kinase (β-ARK1 or GRK2) or a peptide inhibitor of β-ARK1 (β-ARKct). Mice overexpressing β-ARK1 demonstrated attenuation of isoproterenol-stimulated left ventricular contractility *in vivo*, dampening of myocardial adenylyl cyclase activity, and reduced functional coupling of β-adrenergic receptors. Conversely, mice overexpressing β-ARKct showed enhanced cardiac contractility both with or without propranolol (Koch et al. 1995). Moreover, inhibition of β-ARK1 with β-ARKct prevented cardiac dysfunction in several models of heart failure (Rockman et al. 1998, Cho et al. 1999, Harding et al. 2001). Recently, it was demonstrated that the levels of β-ARK1 inhibition determines degree of cardiac dysfunction after chronic pressure overload-induced heart failure (Tachibana et al. 2005).

In addition to overexpression of β-adrenergic receptor, targeted disruption of selective β_1- and β_2-

subtypes have been described. As β_1-subtype mediate the chronotropic and inotropic effects of catecholamines, chronotropic reserve in β_1-deficient mice is markedly limited and heart rate responses to exercise is depressed (Rohrer et al. 1996). In contrast, deletion of β_2-receptor gene did not alter cardiac responsiveness to catecholamines, but altered metabolic response to exercise (Chruscinski et al. 1999).

The pro-apoptotic response to β_1-adrenergic receptor chronic stimulation was recently reinforced by data obtained in selective β_1 and β_2 adrenergic receptor knockout mice (Patterson et al. 2004). β_2-knockout mice (β_1-receptor is the main subtype remained in cardiac myocytes) treated with isoproterenol for 14 days presented an increased mortality rate and cardiac dysfunction and apoptosis, whereas β_1-knockout mice had cardiac function and ultrastructure preserved.

Another very interesting genetic model that recapitulate several aspects of heart failure in humans is based on disruption of α_2-adrenergic receptor in mice. Both α_{2A} and α_{2C} adrenergic receptor subtypes modulate sympathetic tone (MacMillan et al. 1996, Altman et al. 1999), and disruption of both α_{2A} and α_{2C} adrenergic receptors in mice leads to chronically elevated sympathetic tone (Hein et al. 1999). These knockout mice present cardiomyopathy induced by sympathetic hyperactivity and showed reduced exercise capacity, decreased maximal oxygen consumption, decreased cardiac contractility, and significant abnormalities in the ultrastructure of cardiac myocytes (Brum et al. 2002). Considering that most murine models of heart failure are based on the disruption or overexpression of genes for cardiac specific proteins, α_{2A} and α_{2C} knockout mice provide evidence that chronic elevation of sympathetic tone can lead to heart failure in the absence of genetically induced alterations in myocardial structural or functional proteins.

Even though α_1-adrenergic receptors are expressed in a lower level in cardiac myocyte when compared with β adrenergic receptor (ratio of 10:1 for β and α_1), genetic manipulation of α_1-adrenergic receptors has also been demonstrated to affect cardiac structure and function (Simpson et al. 1991). The heart expresses all 3 subtypes of α_1-adrenergic receptors, namely α_{1A}, α_{1B}, and α_{1D}, being α_{1B}-subtype more abundantly expressed in the heart. Transgenic mouse models with overexpression of α_{1B}-adrenergic receptors demonstrated that this subtype might induce cardiac hypertrophy in some but not all transgenic strains (Akhter et al. 1997, Grupp et al. 1998, Lemire et al. 1998, Zuscik et al. 2001). In contrast, deletion of single α_1-adrenergic receptor did not affect cardiac structure or function in resting mice (Cavalli et al. 1997, Rokosh and Simpson 2002, Tanoue et al. 2002). Of interest, mice lacking both α_{1A}- and α_{1B}-adrenergic receptors showed reduced cardiac growth after birth and functional alterations that partly resemble changes observed in heart failure (McCloskey et al. 2003, O'Connell et al. 2003, Turnbull et al. 2003).

G Proteins and Adenylyl Cyclase

As previously mentioned, several G proteins are involved in adrenergic receptors signaling, including G_s and G_i, which modulate AC activity, and G_q that activates phospholipase C. Similar to that observed with adrenergic receptors, genetic manipulation of these downstream signaling components results in a variety of cardiac phenotypes.

Increased levels of inhibitory Gi are widely accepted as a contributor to β-adrenergic receptors dampened signaling in cardiac hypertrophy and failure. Indeed, mice genetically engineered to conditionally express a modified Gi coupled receptor (Ro1) developed lethal cardiomyopathy associated with a wide QRS complex arrhythmia, with a mortality rate greater than 90% at 16 weeks (Redfern et al. 2000). These results are the first to suggest the potential deleterious role for increased Gi expression in the development and progression of heart failure, which contrasts with the notion of Gi signaling being protectant due to its antiapoptotic effects. Thus, more research needs to be performed to elucidate the relative role of increased Gi signaling in

heart failure.

In contrast to Gi, the role of increasing Gs signaling in heart failure has been described in the greatest detail. Transgenic mice overexpressing Gs show baseline tachycardia, enhanced chronotropic and inotropic response to isoproterenol, altered β-adrenergic receptor density, and increased frequency of cardiac arrhythmias (Iwase et al. 1996). Furthermore, overexpression of Gs is associated with myocyte hypertrophy, apoptosis and fibrosis (Iwase et al. 1996, Lader et al. 1998, Geng et al. 1999). These adverse effects seem to be related to enhanced L-type calcium currents in Gs transgenic mice, an effect independent of cAMP pathway.

Transgenic mice overexpressing Gq also exhibit a myopathic phenotype associated with cardiac hypertrophy and fibrosis, which recapitulates that observed in pressure overload hypertrophy (D'Angelo et al. 1997, Sakata et al. 1998). Likewise, transient cardiac expression of Gq led to hypertrophy and dilated cardiomyopathy (Mende et al. 1999). In addition, the decreased cardiac contractility observed in Gq transgenic mice seems to be related to a decreased calcium inflow by L-type calcium channels, and increased Gi. In these models, inhibition of Gi caused sudden death, which suggest that the increased Gi expression might be a compensatory mechanism to counteract other detrimental signaling caused by Gq pathway.

To date, overexpression of more distal components of Gs signaling pathways, specifically ACs' V and VI, does not induce any form of cardiomyopathy (Roth et al. 1999, Tepe and Liggett 1999). Agonist stimulated AC activity is higher in this model; however, this effect does not translate into markedly increased contractility. Furthermore, no evidence of cardiac structure injury was observed.

In summary, generation and characterization of genetically altered mouse models have greatly advanced our knowledge of the molecular mechanisms underlying the pathogenesis of heart failure and provided valuable insights into the identification of the molecular targets for therapeutic development.

β-ADRENERGIC RECEPTOR POLYMORPHISMS IN HEART FAILURE

The central role played by sympathetic nervous system and its receptors in heart failure makes polymorphisms in receptors genes attractive candidates for risk factor and/or predictors of response to treatment.

Polymorphisms of many genes, including of adrenergic receptor signaling pathways and renin-angiotensin system together with environmental factors can markedly influence the progression of cardiac disease. Liggett et al. (Liggett et al. 1998, Liggett 2000b) have demonstrated that adrenergic polymorphism affects not only receptor signaling and sensitivity to pharmacological agents, but also influence clinical outcomes.

For human β_1-adrenergic receptor, two major polymorphic loci have been identified. One of them is a $Ser^{49}Gly$ polymorphism in the extracellular N-terminus (Borjesson et al. 2000). The allelic distribution of $Ser^{49}Gly$ polymorphism has been associated with long-term survival (decreased mortality risk in subjects with Gly^{49}) of patients with heart failure (Borjesson et al. 2000). This finding might be related to results form *in vitro* studies that demonstrated increased desensitization and down-regulation of the Gly^{49} variant (Levin et al. 2002, Rathz et al. 2002), consistent with the idea that β_1-adrenergic receptor blockade or desensitization is protective in heart failure (Bristow 2000). However, contrasting results have been reported in the literature, since Podlowski et al. (Podlowski et al. 2000) found that $Ser^{49}Gly$ polymorphism is more frequent in patients with idiopathic dilated cardiomyopathy.

Another important polymorphism of β_1-adrenergic receptor is an $Arg^{389}Gly$ polymorphism in the 4^{th} intracellular loop, which participates in G-protein coupling. Arg^{389} variant is threefold more effective than is the Gly^{389} variant at activating adenylyl ciclase (Mason et al. 1999). Of interest, the Gly^{389} is considered to be the "wild type" allele since it was cloned first. However, its frequency in Caucasian population is $\sim$0.27 (Liggett 2000b). In

spite of data about this polymorphism, a few trends are apparent. Even though Tesson et al. (Tesson et al. 1999) have observed no direct correlation between $Arg^{389}Gly$ polymorphism and heart failure, more recently Arg^{389} variant, even when expressed at lower levels in mouse hearts, induced heart failure, whereas Gly^{389} variant did not (Mialet Perez et al. 2003). Indeed, responses to antagonists are greater in the Arg^{389} than in Gly^{389} variant (Johnson et al. 2003, Mialet Perez et al. 2003, Sofowora et al. 2003).

α_{2C} - adrenergic receptor polymorphism has uncovered a significant increased risk of progression to heart failure, particularly in African American subjects, when the gain of function Arg^{389} β_1-adrenergic receptor polymorphism is associated with the loss of function α_{2C}-adrenergic deletion variant (decreased inhibition of noradrenaline release from sympathetic nerve terminals, which is consistent with findings of α_{2A}/α_{2C} adrenergic receptor knockout mice) (Small et al. 2002). Of interest, Arg^{389} β_1-adrenergic receptor polymorphism has not been associated with increased cardiovascular risk by itself; however it significantly increases the cardiovascular risk of α_{2C}-adrenergic deletion variant. These results highlighted the importance of considering the combinations of individual polymorphisms, which results in several haplotypes that have not been investigated in detail.

Overall, as recently reviewed by Michel and Insel (Michel and Insel 2003), inconclusive results have been obtained regarding β_1-adrenergic receptor polymorphisms. Further work is need to define the role of these polymorphisms play in heart disease and drug response, perhaps as haplotypes (Kirstein and Insel 2004, Lohse 2004).

Although β_2-adrenergic receptors are expressed in the heart at lower concentrations than are β_1-subtype, they are more numerous in many other sites, including vascular, bronchial, gastrointestinal smooth muscle, glands, leukocytes, and hepatocytes; and more importantly β_2-adrenergic receptors are highly polymorphic. One rare and never homozygous $Thr^{164}Ile$ variant was associated with reduced survival and depressed exercise capacity in patients with heart failure (Liggett et al. 1998, Wagoner et al. 2000, Brodde et al. 2001). These results are consistent with findings in transgenic mice with $Thr^{164}Ile$ polymorphism targeted to the heart (Turki et al. 1996).

Another polymorphism associated with heart failure is $Gln^{27}Gly$ variant in β_2-adrenergic receptor. It has recently been reported that heart failure patients homozygous for the Gln^{27} allele were less likely to respond to the β-blocker carvedilol compared with Glu^{27} allele (Kaye et al. 2003). This result suggests that Gln^{27} influences the responsiveness of heart failure patients to β-blocker therapy. In addition, we have recently reported that humans with polymorphism of β_2-adrenergic receptor at codons 16 and 27, namely women who are homozygous for Arg^{16}/Glu^{27} haplotype, have augmented muscle vasodilatory response to mental stress and exercise (Trombetta et al. 2005). Whether this result will be reproducible in heart failure patients is not known.

In summary, the contribution of β_1 or β_2 adrenergic receptor polymorphisms for progression of heart failure still need to be better investigated. Prospective studies of sufficient size are lacking, as well as, studies designed to consider the many complex haplotypes comprising a combination of individual polymorphisms.

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RESUMO

A insuficiência cardíaca (IC) é a via final comum da maioria das doenças cardiovasculares e uma das maiores causas de morbi-mortalidade. O desenvolvimento do estágio final da IC freqüentemente envolve um insulto ini-

cial do miocárdio, reduzindo o débito cardíaco e levando ao aumento compensatório da atividade do sistema nervoso simpático (SNS). Existem evidências de que apesar da exposição aguda ser benéfica, exposições crônicas a elevadas concentrações de catecolaminas, liberadas pelo terminal nervoso simpático e pela glândula adrenal, são tóxicas ao tecido cardíaco e levam a deterioração da função cardíaca. Em nível molecular observa-se que a hiperatividade do SNS está associada a alterações na sinalização intracelular mediada pelos receptores beta-adrenérgicos. Sabe-se que tanto a densidade como a função dos receptores beta-adrenérgicos estão diminuídas na IC, assim como outros mecanismos intracelulares subjacentes à estimulação da via receptores beta-adrenérgicos. Nesta revisão, apresentaremos uma breve descrição da via de sinalização dos receptores beta-adrenérgicos no coração normal e as conseqüências da hiperatividade do SNS na IC. Daremos ênfase ao potencial miopático de diversos componentes da cascata de sinalização dos receptores beta-adrenérgicos discutindo estudos realizados com animais geneticamente modificados. Finalmente, discorreremos sobre o impacto clínico do conhecimento dos polimorfismos para o gene do receptor beta-adrenérgico para um melhor entendimento da progressão da IC.

Palavras-chave: insuficiência cardíaca, sistema nervoso simpático, receptores adrenérgicos.

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